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JOURNAL OF ANATOMY

PHASES OF MATURATION AND FERTILIZATION IN HUMAN OVA

By W. J. HAMILTON, *Department of Anatomy, St Bartholomew's Hospital Medical College, London*

During the past year it has been possible, with the co-operation of Miss G. H. Dodds, M.D., F.R.C.S., and Miss Josephine Barnes, M.D., F.R.C.S., of University College Hospital, London, to secure two living human ova from cases under their care.

The first specimen, ovum no. 1, at the stage of the second maturation spindle, was secured from a woman (Mrs G.) aged 29 years who had previously had two children. As she was suffering from severe mitral stenosis it was considered desirable that she should be sterilized. She stated that her menstrual cycle was of the 28-day type and that menstruation lasted 2 days. The cycle, however, previous to the one in which the ovum was recovered was of 30 days' duration; the first day of that cycle was 18 May 1942 and the next menstrual cycle began on 17 June. The operation was performed on 3 July 1942 at 2 p.m., i.e. on the 17th day of the cycle or 14 days before the estimated onset of the next cycle assuming that the cycle from which the ovum was secured was of the 30-day type. On the evening before operation the right ovary, on vaginal examination, was found to be enlarged and slightly tender. At operation, when the ovaries were examined, the left ovary was normal; the right ovary showed that a follicle had recently ruptured and a small amount of blood was escaping from the opening of the collapsed follicle. A bilateral salpingectomy was performed.

The second specimen, ovum no. 2, at an early stage of fertilization, was obtained from a woman (Mrs S.) aged 40 who had had five previous pregnancies, the last two of which were associated with severe toxaemia and terminated as miscarriages, the last of which occurred in February 1942. On account of her clinical condition it was considered advisable that she should be sterilized. Previous to her last miscarriage her menstrual cycles were of the 28-day type, menstruation lasting 6–7 days. Her last menstrual period before the operation commenced on 16 April 1942. Coitus took place on 27 and 29 April and the operation was performed at 9.30 a.m. on 1 May, i.e. on the 16th day of the cycle. On opening the abdomen a small amount of free fluid was found in the peritoneal cavity. The left ovary was normal. The right ovary showed a recently ruptured follicle from the opening of which a small amount of blood was escaping. There was also a cyst in this ovary. A bilateral salpingectomy and right oophorectomy were performed.

TECHNIQUE

After the uterine tubes were removed at operation they were tied at both ends and placed in Locke's solution until they were taken to the laboratory. On arrival there, the mesosalpinx and peritoneum were removed from the tubes so as to prevent kinking and to allow a free flow of fluid to pass during the subsequent flushing with Locke's solution which was done from the uterine to the fimbriated end into watch-glasses. In each case the ovum was recovered in the first few c.c. of the washings from the tube corresponding with the ovary from which ovulation had occurred. The ova were photographed, while presumably still living, with a Vickers Projection Microscope at a magnification of 480 diameters. They were fixed by the gradual addition of Bouin's Fluid (Allen's modification, P.F.A. 3) to the Locke's solution. Dehydration was carried out in the usual way. They were double embedded in celloidin and paraffin. Unfortunately, during these manipulations the second specimen was lost. Complete serial sections at 8 μ were cut of the first ovum by Mr E. Park.

DESCRIPTION

First specimen. When examined in the fresh state several layers of loosely arranged cumulus cells were found attached all around the zona pellucida which had a homogeneous appearance and was closely applied to the vitellus (Pl. 1, fig. 1). Owing to the presence of the cumulus cells the ovum did not roll freely in the watch-glass and at no time was a polar body visible nor could any details of nuclear material within the ovum be recognized. The light yellowish, uniformly granular vitellus completely filled the zonal cavity. There were no such subdivisions of the yolk material as described by Lewis (1931). The dark areas shown in the photograph are due to the presence of cumulus cells on the upper or lower surface of the zona.

After fixation and dehydration many of the follicle cells were dispersed so that in the sectioned ovum relatively few are attached to the zona. With fixation the vitellus underwent a shrinkage greater than that of the zona pellucida so that a perivitelline space appeared (Pl. 1, fig. 2). After staining with modified Harris's haematoxylin and eosin the cytoplasm appears uniformly granular and shows no evidence of polarity (Pl. 2, figs. 5, 7). The 2nd maturation spindle, cut slightly obliquely, is present at the stage of metaphase. The first polar body shows scattered chromosomes in its cytoplasm (Pl. 2, figs. 6, 7).

Second specimen. When examined in the fresh state this was found to be free of cumulus cells. The zona pellucida appeared as a homogeneous membrane in which many complete sperms could be seen. Some were lying obliquely, others transversely

(Pl. 1, figs. 3, 4). The vitellus did not completely fill the zonal cavity; it was coarsely granular, and at one depth of focus showed, in a clearer zone towards the centre, what may have been the spindle of the 1st cleavage. The general appearance of the vitellus, however, is that of early degeneration. Between the vitellus and the zona at one side a number of globules are seen which are probably the remains of the disintegrating polar bodies. It was unfortunate that it was not possible to verify the structures seen in this ovum by subsequent sections.

The measurements of both ova, together with other human ova, are given in Table 1.

DISCUSSION

Allen, Pratt, Newell & Bland (1930) give an account of the attempts that have been made to secure human tubal ova. They succeeded in obtaining five ova by reverse irrigation of the uterine tube from the uterus. The photographs of the living eggs found by them are unsatisfactory and the histological details leave much to be desired. Undoubtedly some of the ova are undergoing degenerative changes. Lewis (1931) obtained an unfertilized ovum by flushing the uterine tube which had been previously removed with a myomatous uterus. The vitellus was dark and everywhere in contact with the zona pellucida and showed three types of yolk bodies which were irregularly distributed in it. Two further ova have been recovered by Pincus & Saunders (1937). One of these ova contained a dark coarse cytoplasm with several large dark yolk particles, similar to those of the Lewis ovum, and the vitellus was in close contact with the zona pellucida. This egg was cultured and the movements that occurred in the cytoplasm were apparently of a degenerative nature. The second ovum contained no dark yolk masses. It was entangled, however, in a mass of cells which were thought to represent the remains of the cumulus oophorus and which obscured the details of the cytoplasm in the photograph. It was thought that this ovum had only recently been shed.

In ovum no. 1 of the present account, at the second maturation spindle stage, the cytoplasm is uniformly granular and thus more closely resembles the second ovum of Pincus & Saunders than any so far described. This uniformity of cytoplasm is in contrast to that described by Allen *et al.* (1930) who found that the central cytoplasm of the vitellus was coarsely granular and the peripheral 'exoplasm' was finely granular. It seems probable that ovulation had occurred recently. Histologically this ovum shows no degenerative changes. Rock & Hertig (1942) report the isolation of a 'perfect unfertilized tubal ovum' but give no details concerning it.

As can be seen from Table 1 there is considerable variation in the size of the different ova described, the smallest being that described by Pincus & Saunders (1937) and the largest ovum no. 2 of the present series.

Maturation. There is a comprehensive literature on the maturation of the oocyte in different mammals, but up to the present only a few cases have been described in the human subject.

In all mammals so far examined, with the possible exception of the dog (Van der Stricht, 1923), the second maturation division begins in the ovary but is not completed until after fertilization. The first description of the formation of the first and second polar bodies in man was given by Thomson (1919) and was based on examination of follicles which undoubtedly show either atretic or degenerative changes and, therefore, need not be considered further. He believed that the second polar body was formed when the ovum was still in the ovary.

The first maturation stage of the human oocyte was described by Stieve (1926). He found a primary ovarian oocyte with two distinct masses of nuclear material close to the periphery. A further stage of the first maturation was described by Hoadley & Simons (1928); the nucleus was at the 'mesophase'. Recently, Rock & Hertig (1942) report having recovered two oocytes at the first maturation division from ovarian follicles. Dixon (1927) described an oocyte in the ovary at the second maturation spindle stage and concluded, without sufficient evidence, in my view, that the second polar body is formed before the oocyte leaves the ovary and before fertilization has taken place. The appearances seen in ovum no. 1, which is at the metaphase and is already shed, and the description given by Allen *et al.* (1930) for two of their shed ova, and also by Pincus & Saunders (1937) do not support the opinions advocated by Dixon. There is no doubt that the second maturation spindle persists until after ovulation, but the period of time that the oocyte remains at the second maturation division stage, or whether it is completed in the absence of fertilization, cannot be stated until more material is available.

Time of ovulation. The recovery of ovum no. 1, on the 17th day of the menstrual cycle in a woman who stated that normally her cycle was of 28 days, although the previous cycle, which was accurately timed, was of 30 days' duration, and of ovum no. 2, on the 16th day of the cycle in a woman who had a cycle of 28 days, raises the question of the time of ovulation.

It is probable that ovum no. 1 had only recently been shed less than 12 hr. previously (see clinical history and findings on vaginal examination). The cumulus cells are still present, as they are in some other ova described (see Table 1), and the specimen is well fixed and stained and shows no degenerative changes. From the comparative aspect it is worthy of note that Pincus (1930) found, in the rabbit, that

Table 1. *The details of this table are based on data given in the text or in the tables of the authors quoted*

Ovum	Measurements in μ: Including zona pellucida	Vitellus alone	Zona pellucida (thickness)	Corona cells	Polar bodies	Day of cycle ovum recovered	Length of menstrual cycle	Presumed no. of days between ovulation and onset of next cycle	Remarks
Allen *et al.*:									
(1)	184 × 151	109 × 100	16–18	Nil	2	15th	Not stated	—	Ovum lost
	193 × 151	107 × 100	20	—	(19μ)	—	—	—	
	—	102 × 102	Swollen	—	—	—	—	—	
	(Measured at 20 min. intervals)								
(2)	134 × 131	—	Not apparent	Present	1	15th	Not stated	—	
(3)	132 × 132	102 × 102	6 (fixed)	Partly dispersed	1	15th	Not stated	—	
	(Twin of no. 2—one from each ovary)								
(4)	147 × 135	140 × 93	—	Partly dispersed	1	16th	Not stated	—	
(5)	Fragmented	138 × 90	—	3 layers	—	14th	Not stated	—	
Lewis	151 × 145	138 × 133	6	Nil	—	22nd	Formerly 28, last 35	14–15	Ovum probably 1 day old
Pincus & Saunders:									
(1)	125 × 115	110 × 95	7–10	Nil	—	17th	28	12	Ovum probably 2 days old
(2)	121 × 121	111 × 111	5	Nil	1	20th	26–30	9–13	Ovum probably 2 days old
Present paper:									
(1)	(Fresh) 133	106	13	Present	1	17th	Formerly 28, last 30	14	Ovum not more than 12 hr old
	(Fixed) 115	83	—	—	—	—	—	—	
		77 (section)							
(2)	171	133	19	Nil	? Polar debris	16th	28	14–15	Ovum lost—probably 1 day old

the cumulus cells persisted for 10 hr. after ovulation in unfertilized eggs and only for about 5 hr. in fertilized ones. In the mouse (Lewis & Wright, 1935) in both fertilized and unfertilized eggs the cumulus cells disappear at about 12 hr. after ovulation.

Ovum no. 2 was probably shed at least one day previously, all the cumulus cells having disappeared and many sperms being found in the zona pellucida. There is just a possibility that this egg had been fertilized; in a clear area towards the centre there are dark rod-like bodies suggestive of chromosomes on the spindle of first cleavage (cf. Pincus, 1930), in the rabbit). The recovery of these eggs shows that ovulation occurs, in women with a 'regular' cycle, at approximately the middle of the menstrual cycle.

On the subject of the time of ovulation in the menstrual cycle there is an extensive literature which has been fully reviewed by a number of investigators (see Knaus, 1934; and others). Evidence, however, is accumulating which seems to show that ovulation is more closely related to the succeeding menstrual period than to the beginning of the preceding one (see Knaus, 1934; Ogino, 1934; Pryde, 1941; and Smith, 1942). Rock & Hertig (1942), by comparing the ages of young embryos with the associated endometrial histology in seven cases, and especially in two which were well controlled, believe that ovulation occurs 14 (15 to 13) days before the anticipated date of the next menstrual period. Further, if the menstrual histories of the cases from which human ova have been recovered are analysed the data tend to show that ovulation is more closely related to the beginning of the succeeding period. The Lewis ovum (1931) was recovered on the 22nd day of the cycle. As ovulation had probably occurred 24 hr. earlier and assuming that this cycle would have been of 35 days' duration, as was the preceding one, then ovulation occurred 15 days previous to the beginning of the succeeding cycle. In ovum no. 1 of Pincus & Saunders (1937), ovulation is estimated to have occurred 12–14 days before the beginning of the next cycle, and in their ovum no. 2, 9–13 days before the 1st day of the next menstrual period, depending upon whether the present cycle was of 26 or 30 days' duration. The menstrual histories of the two cases from my series described in this paper also indicate that both ovum no. 1 and ovum no. 2 were ovulated 14–15 days before the presumed beginning of the next menstruation.

SUMMARY

1. A description is given of two human tubal ova.

2. Ovum no. 1 in the living state was surrounded by cumulus cells. The vitellus completely filled the zonal cavity.

3. On section this ovum was found to be at the metaphase of the second maturation division.

4. Ovum no. 2 showed spermatozoa in the zona pellucida.

5. The time of ovulation in women is discussed.

I wish to acknowledge my indebtedness to the Publications Fund of the University of London for a grant towards the cost of the coloured illustration of this communication. I also wish to thank Mr A. K. Maxwell for the *natur-treu* figure; and also Mr A. E. Westwood for the photographs.

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EXPLANATION OF PLATES

The photomicrographs have been reproduced without retouching.

PLATE 1

Fig. 1. Photomicrograph of ovum no. 1 in the living state. The dark areas on the vitellus are cumulus cells on the upper or lower surface of the zona. ×480.

Fig. 2. The same ovum immediately after fixation. ×480.

Fig. 3. Photomicrograph of ovum no. 2. A complete sperm is seen in the zona pellucida; other sperm heads are visible. At the lower left hand corner of the photograph globular detritus is seen. ×480.

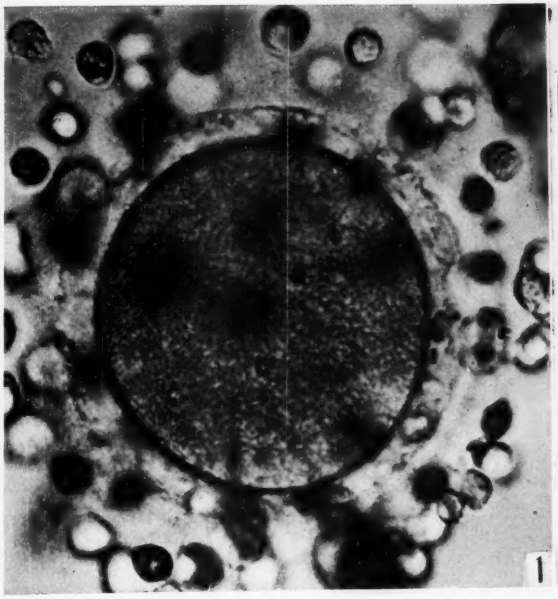
Fig. 4. Photomicrograph of the same egg at a different focus. In a clearer area towards the centre of the vitellus rod-like bodies (? chromosomes) are seen. ×480.

PLATE 2

Fig. 5. A section of ovum no. 1 showing the metaphase of the second maturation division. The first polar body (out of focus in the lower left hand corner) is seen. The vitellus has shrunk from the zona pellucida and most of the cumulus cells have been cast off (cf. Pl. 1, fig. 1). ×840.

Fig. 6. Photomicrograph of part of same section as in fig. 5 with the polar body in focus. ×840.

Fig. 7. A drawing of same section as that in figs. 5 and 6 showing the details of the second maturation spindle and the state of the 1st polar body. The cytoplasm is seen to be uniformly granular. ×840.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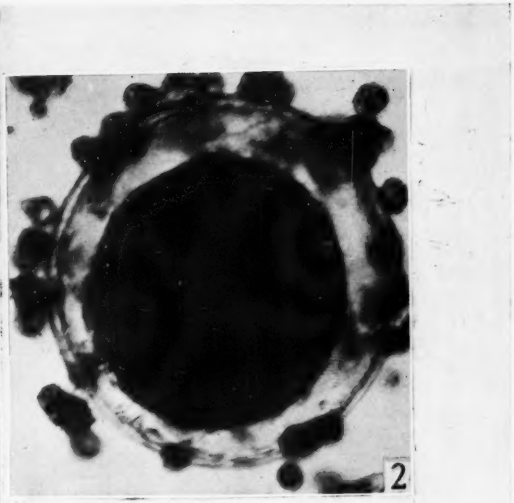
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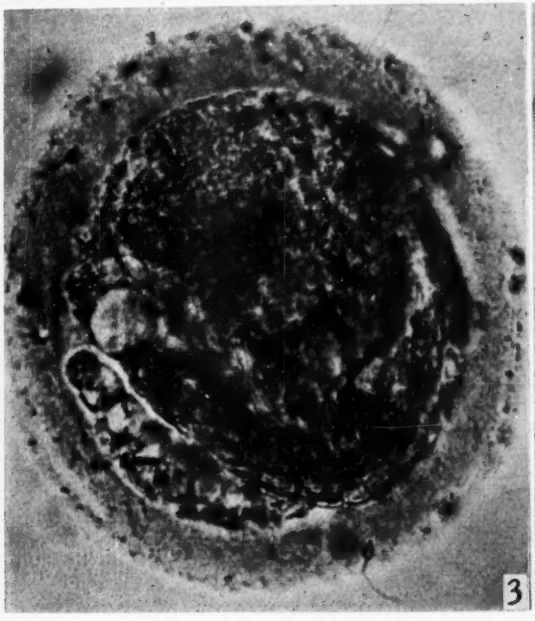
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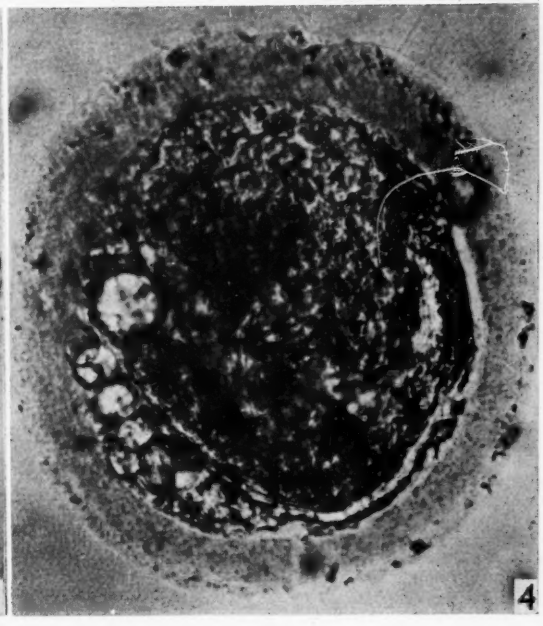
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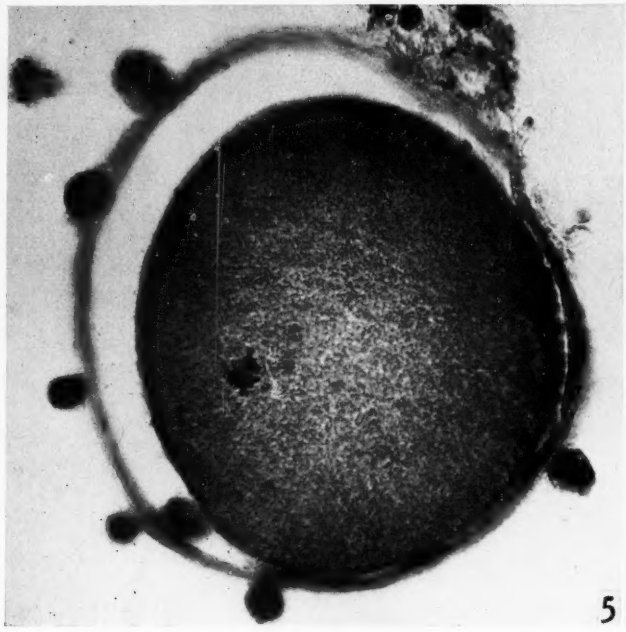

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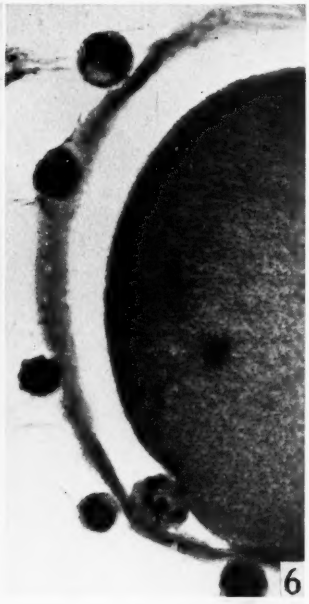

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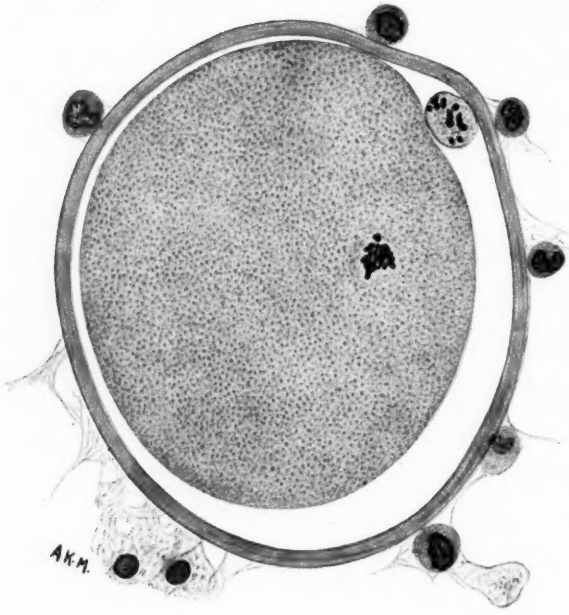

7

HAMILTON—PHASES OF MATURATION AND FERTILIZATION IN HUMAN OVA

TRANSUTERINE ('INTERNAL') MIGRATION OF THE OVUM IN SHEEP AND OTHER MAMMALS

By J. D. BOYD,* W. J. HAMILTON† and J. HAMMOND, Jr.‡

In the course of investigations on the genital tract of pregnant sheep, ova, blastocysts and embryos of known age were found in positions that appear to throw some light on the problem of the transuterine ('internal') migration of the 'ovum'.§ In examining the literature it was found that while the subject of migration of the ovum has been dealt with by a number of investigators, particularly Corner (1921), no complete review was available. In addition, many investigators have implied, in their data, the occurrence of migration without having stated explicitly that it had taken place.

The term internal migration is generally used to describe the passage of the ovum (or its derivatives, the blastocyst or chorionic sac) from one uterine horn to the other by way of a common uterine body in those species with a bicornuate uterus. External migration is the term used to describe the phenomenon of the passage of an ovum shed from one ovary across the peritoneal cavity to the ostium of the opposite uterine tube and thence to the opposite uterine horn. Fränkel (1924) suggested that the term uterine migration should be used for the phenomenon of internal migration and peritoneal migration for external. We, however, prefer the terms transuterine and transperitoneal for the two types of migration.

We wish to thank Dr John Hammond, F.R.S., for additional data which make our conclusions on the frequency and distribution of corpora lutea and embryos in the sheep more significant.

An account is given of our own observations in the sheep, and this is followed by a review which shows that transuterine migration is of widespread occurrence in Eutheria.

TRANSUTERINE MIGRATION IN SHEEP

Most of the sheep were of mixed or undetermined breed; the uteri were collected and examined in most cases within 15 min. of the death of the animal thanks to the co-operation of the slaughterhouse authorities. In a number of cases (Table 1) the side on which ovulation occurred is known; in many cases, however, it was known that two ovulations had occurred from one ovary but the side was not recorded. A correlation between ovary and uterine horn was, of course, available.

Table 1 and Text-fig. 1 summarize the distribution of corpora lutea and the number and situation of pregnancies in the seventeen cases of multiple ovulation in the series which we have examined specifically for evidence of migration. It will be seen that in five of the sheep two corpora lutea were found in one ovary while the resulting conceptuses were found one in each uterine horn (Text-fig. 1 C). These included one pair of twins of about 20 days (late somite stages, 3·5 mm. long) and four pairs of twin embryos varying between 12 and 40 mm. c.r. length.

In four sheep of this series there were two ovulations in a single ovary with both the resulting conceptuses in the corresponding horn. These included one pair of embryos in which there was a normal embryo of 26 mm. c.r. and a 6 mm. degenerated abortus. The other three cases were 10-, 12- and 13-day blastocyst twins (Text-fig. 1 B).

In two sheep two recently ruptured follicles were found in one ovary, and in each of these cases two unfertilized ova were washed out of the corresponding tube (Text-fig. 1 A).

In two sheep two corpora were found in the left ovary, but in each case a single conceptus was found in the right uterine horn. In each case the embryo was about the 30 mm. stage (Text-fig. 1 E).

In one sheep two corpora were found in the right ovary and two 19-day embryos were found in the right horn, but their chorionic sacs extended through the unpaired 'body' into the other uterine horn (Text-fig. 1 D). In one sheep there were two corpora in the right ovary, but only one embryo, of about 20 days' gestation, was found in the right horn though its chorionic sac extended, as in the previous case, into the left horn. Finally, in two sheep two corpora were found in the right ovary and there was one embryo in the right horn and one in the unpaired body of the uterus (Text-fig. 1 F). These were both comparatively late pregnancies, the embryos measuring about 16 cm. c.r. length. The left horn in each case was much smaller than the right and the uterine body was larger than normally found in twin pregnancies. In each of these sheep the chorions of the embryos had fused to form a single sac

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§ Theoretically, the term ovum should not be used after the commencement of cleavage. By long-established practice, however, the term is loosely applied to the early developing embryo.

and, in one, the amnions were in contact and firmly united but the amniotic cavities were separate.

In the series just described, in which there was multiple ovulation in one ovary, the conditions found can be summarized as follows: (1) ova in the corresponding tube; (2) two blastocysts in the corresponding uterine horn; (3) older embryos situated one in each horn; and (4) intermediate stages, as demonstrated in Text-fig. 1 F.

It seems noteworthy that in the one case where two embryos at a relatively advanced stage were found in a single horn one was atrophic.

In all the other more advanced twin pregnancies the embryos were either one in each horn or one in one horn and the other in the common uterine body. Whether one of the two embryos in Text-fig. 1 B would have migrated at a later stage to the body or opposite horn, or have undergone atrophy, must remain a matter for speculation.

In addition to the above series in which the age or size of the conceptuses are known within narrow limits data are also available, through the courtesy of Dr J. Hammond, F.R.S., on a much larger series of multiple pregnancies which show that migration of embryos from one horn to the other must have occurred. In this series of seventy-eight pregnant sheep there were forty-six with a single corpus luteum in one ovary and a pregnancy in the corresponding horn. There was only one sheep with a single corpus luteum in one ovary and a pregnancy in the opposite horn. This appears to suggest that migration (transperitoneal or transuterine) is unusual in single pregnancies in the sheep. In fifteen sheep with two corpora lutea in one ovary one embryo was found in each uterine horn and consequently migration must have occurred. In twelve sheep there was a single corpus luteum in each ovary with a pregnancy in each horn. In one sheep only were there two corpora lutea in one ovary and two embryos in the horn of the same side. In no case with a single corpus luteum in each ovary were there two embryos found in one horn. One case of triple ovulation showed three corpora lutea in a single ovary and only one pregnancy in each horn, the third ovum presumably not having been fertilized or the resulting embryo having degenerated. Another case of triple ovulation had two corpora lutea in one ovary and one in the other and a corresponding arrangement of the embryos in the uterine horns. In one case in which four corpora lutea were found, one in the right ovary and three in the left, only one embryo was found in each horn.

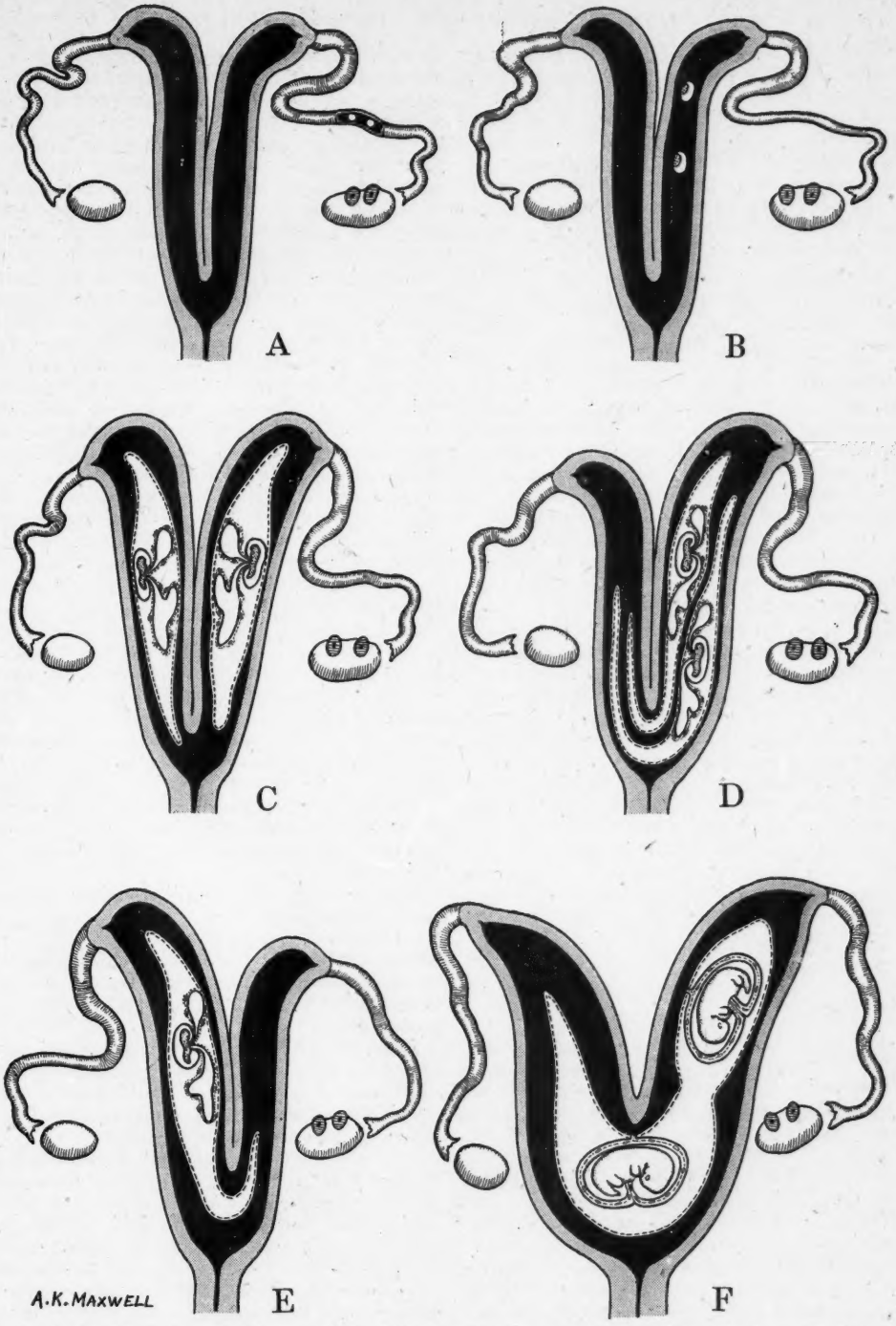
The fifteen cases in which there were two ovulations from a single ovary, and a distribution of the embryos one to each horn, provide further convincing evidence of the reality and frequency of migration in the sheep, for in only one case were two corpora lutea in a single ovary associated with two embryos in a corresponding horn. Further, the fact that migration was not observed in embryos with a single ovulation from each ovary suggests that the distribution is not random but appears to

Table 1. *Data for transuterine migration in double ovulations*

Sheep no.	Ovulations	Pregnancies	State of development
W. 1	2 in right ovary	1 in each horn	25 mm. embryos
W. 4	2 in right ovary	1 in each horn	40 mm. embryos
N. 12	2 in one ovary	1 in each horn	12 mm. embryos
N. 16	2 in one ovary	1 in each horn	14 mm. embryos
N. 26	2 in one ovary	1 in each horn	20 day embryos
Sp. 9	2 in left ovary	2 in left horn	12 day blastocysts
N. 31	2 in one ovary	2 in horn of same side	One 26 mm. embryo and one 6 mm. abortus
Sp. 4	2 in one ovary	2 in horn of same side	13 day chorionic sacs
Sp. 8	2 in one ovary	2 in horn of same side	10 day blastocysts
N. 17	2 in one ovary	2 unsegmented ova in tube corresponding with ovary in which ovulations had occurred	
N. 32	2 in one ovary	2 unsegmented ova in tube corresponding with ovary in which ovulations had occurred	
W. 2	2 in left ovary	1 in right horn	30 mm. embryo
W. 3	2 in left ovary	1 in right horn	30 mm. embryo
Sp. 15	2 in right ovary	2 in right horn (chorionic sacs extended into left horn)	19 day embryos
W. 5	2 in right ovary	1 in right horn (chorionic sac extended into left horn)	20 day embryo
W. 6	2 in right ovary	1 in right horn 1 in fused horn region	160 mm. embryos*
W. 7	2 in right ovary	1 in right horn 1 in fused horn region	160 mm. embryos†

* The umbilical cords of both embryos were attached to the medial side of the right horn, the lower close to the junction with fused horn. The right horn was much larger; the left horn gradually tapered from the fused horn region. The amnions were fused.

† Both embryos were attached to the medial side of the right horn. The amnions were not fused but the chorions were.



Text-fig. 1. Schematic representations of the relationships between two ovulations from a single ovary and the distribution of the resulting conceptuses as found in our series of sheep. (For details see text.)

correspond to the spacing of eggs in polyovular mammalian species.

Although transperitoneal migration is possible in the sheep, since the ovaries are not in a closed ovarian bursa, the data in Table 1 are strongly in favour of transuterine migration. Further, in all those sheep in which there was a single ovulation the conceptus, with one exception, was found in the same tube or uterine horn as that of the ovary from which ovulation occurred.

STRUCTURE OF THE UTERUS IN THE SHEEP

The uterus in the sheep is a typical ungulate bicornuate uterus with two horns, a short common body and a cervix. To the naked eye the appearance of the mucosa of the body is very similar to that of the horns, possessing typical caruncles (cotyledons) with melanin-like pigmentation. Detailed histological studies have been made on the genital tract of the sheep at different stages in the oestrous cycle by Casida & McKenzie (1932), Cole & Miller (1935) and McKenzie & Terrill (1937). None of these investigators, however, have specifically stated that the endometrium of the body is identical in structure with that of the cornua. As the chorion comes into intimate and, presumably, functional contact with the mucosa of the corpus uteri we have examined its microscopic structure and compared it with that of the cornua. The appearances are essentially similar both in intercaruncular (cf. Pl. 1, figs. 1, 2) and caruncular regions. A typical caruncle of the body is shown in Pl. 1, fig. 3. The lining of the cervix is very different; glands are almost completely absent and the mucosa is raised up into complicated villous folds (Pl. 1, fig. 4).

In view of the possibility that cilia may be involved in the intra-uterine transference of ova we examined the endometrium with this point in mind. No cilia were apparent.

DISCUSSION

Migration of ova in eutherian mammals

Bischoff (1842) drew attention to the fact that the number of corpora lutea usually corresponds with the number of embryos, but in the rabbit he occasionally found, especially in the later stages, fewer embryos than corpora lutea. This he explained by the abortion of some of the embryos. This has been confirmed by many investigators since (see Hammond, 1921, 1941, for literature). Bischoff pointed out that in the rabbit as a rule just as many 'eggs' are found in each tube or horn of the uterus as there are corpora lutea in the corresponding ovary. In dogs, however, he made the important observation that ova are occasionally distributed in the uterine horns in such a fashion as to be explained only by 'Ueberwanderung des Eies' (migration) from one uterine horn to the other as the number of corpora lutea in each ovary did not correspond with the number of the embryos in the horn. In his paper on the early development of the dog (1845) Bischoff gave more details on this 'wandering' and commented on the possible method by which eggs could be moved from one uterine horn to the other. He concluded that it was most likely due to uterine contractions. He also considered the possibility that the number of corpora lutea in the ovary may be no certain criterion of the number of eggs that have been shed, since there may have been pluriovular follicles and some eggs may have been aborted. He concluded, however, that such an explanation is extremely unlikely in view of the need for the coincidence required to make the number of aborted ova on one side exactly balance the excess on the other; he considered it much more likely that migration occurred under the same (unknown) control that arranges the spacing of the eggs in one horn of the uterus in the dog or other polyovular animals in such a way that they have proper room for development. His conclusion on 'Ueberwanderung des Eies' in the dog is that it is 'eine sehr bemerkenswerthe, aber sicher ausgemachte Thatsache'. In later papers on the development of the guinea-pig (1853) and the European roe-deer (1854), Bischoff also described migration of ova. Leuckart (in Bergmann & Leuckart, 1857) confirmed Bischoff's findings in the guinea-pig, but Reichert (1861) contradicted this and doubted very much if the phenomenon occurred in any mammal. Bischoff (1842, 1859—in a letter to Kussmaul, p. 318) himself never found migration in the rabbit, which he attributed to the fact that here there are completely separated uteri opening separately into the vagina.

Since Bischoff's time several investigators have made specific contributions to the subject of migration of the ovum, and a number of others have referred incidentally to it or have presented data from the examination of which its occurrence or not may be deduced. The contributions of these workers are summarized in Table 2.

Migration of the egg can either be from the ovary to the opposite uterine tube in those animals in which there is no completely closed bursa ovarica (so-called external or transperitoneal migration) or it may be from one horn of a bicornuate uterus to the other by way of a longer or shorter common uterine 'body' (internal or transuterine migration). External migration is theoretically possible in all mammals without an ovarian bursa or with the latter in communication with the peritoneal cavity. A number of cases have been recorded for man (see Kussmaul, 1859; Sippel, 1901; and Grosser, 1927); Sippel, indeed, has emphasized the frequency of the contra-lateral situation of the corpus luteum in

Table 2. ***Transuterine* ('*internal*') *migration in mammals***

Type	Observer (names of observers who specifically describe migration are italicized)	Frequency	Remarks
UNGULATES			
Pig	*Corner*	50% of cases	See footnote 1
	Hausmann	? 1 case in 17	—
	Kelly	—	Experimentally
	Kupfer	7 cases in 29	—
	Warwick	199 cases in 469	Also experimentally
Sheep	*Bonnet*	3 cases in 118	—
	Curson	2 cases in 5	Double pregnancies
	Curson	2 cases in 36	Single pregnancies
	Hausmann	1 case in 16	—
	Present data	—	—
Goat	*Quinlan*	1 case (triple pregnancy)	Quoted by Curson
	Tsukaguchi	1 case, at least, in 14	—
Cow	Bergmann	1 case in 50	See footnote 2
	Day	1 case in 1000	Personal communication
	Kaltner	0 cases in 300	—
	Kupfer	1 case in 1000	See footnote 2
Horse	*Day*	5 cases in 7	—
	Kedrov	—	See footnote 3
Deer (*Capreolus capreolus*)	*Bischoff*	—	—
Procavia capensis	Turner	—	—
	Wislocki	4 cases in 12	—
CARNIVORA			
Dog	*Bischoff*	Observed several times	—
	Bonnet	1 case in 12	—
	Hausmann	2 (? 3) cases in 17	—
	Sparapani	—	See footnote 4
	Spiegelberg	—	Quoted by Kussmaul (p. 319)
Cat	*Hill & Tribe*	1 case in several hundred	—
Ferret	*Hammond & Marshall*	1 case	Experimentally
Badger	*Fischer*	—	Internal migration considered possible
Fox	Personal observation	1 case	—
CHEIROPTERA			
Vespertilio murinus	*Duval*	—	The blastocysts of these bats always imbed in the right horn although there is ovulation from either ovary. Hence migration must occur
Vesperugo pipistrellus	*Duval*	—	
Rhinolophus ferrum equinum	*Duval*	—	
Molossus crassicaudatus	Hamlett	—	
Vesperugo murinus	*Van der Stricht*	—	

(In *Glossophaga soricina* (Hamlett), where the uterus is simplex, and in *Cynopterus marginatus* (Keibel), where the uterine horns open separately into the vagina, migration has not been observed)

Footnotes. (1) In 1915 Corner considered external migration more probable; in 1921 he produced evidence making internal migration a much more likely explanation.

(2) In the cow no records have been made of the migration in twins. Bergmann in two cases and Kupfer in seventeen, in none of which both ovulations were in the same ovary, failed to find migration. Lillie (1917 and 1923) gives no data on corpora lutea in his extensive study of free-martin twins in cattle.

(3) Kedrov, by rectal examination of 574 mares during 804 oestrous cycles, found that ovulation occurs 15–20% more often in the left ovary. As pregnancies were observed more frequently (25–30%) in the right uterine horn he concluded that intrauterine migration must occur.

(4) Quoted by Schmaltz (p. 233) as having found external (peritoneal) migration by experiments comparable to those of Leopold on the rabbit.

Table 2 (*continued*)

Type	Observer (names of observers who specifically describe migration are italicized)	Frequency	Remarks
Insectivora			
Sorex araneus Linn.	*Brambell*	28 cases in ♀ 99	—
Sorex minutus Linn.	*Brambell*	13 cases in ♀ 47	—
Talpa	*Personal observations*	—	Not possible to correlate corpora lutea with pregnancies
Erinaceus	*Deanesly*	—	Not possible to correlate corpora lutea but embryos are fairly evenly distributed between the two horns
Primates			
Lemur rufipes	Turner	1 case in 4	—
Rodentia			
Rabbit	*Leopold* *Asdell* *Baur*	*External migration only*	Migration denied by *Bischoff* and, experimentally, by *Parkes*
Guinea-pig	*Bischoff* *Leuckart*	— —	Migration denied by *Reichert, Squier, Kinney, Nicol* and *Kelly*
Albino rat	*Hanson & Boone* *Slonaker*	No evidence for migration No evidence for migration	— —
Field vole	*Brambell*	No evidence for migration	—

tubal pregnancy and explains the occurrence of the condition by the fact that the time necessary for transperitoneal migration results in the egg reaching the opposite tube at a time when it is ready for implantation. Williams (1941) also has shown that in a considerable number of cases of tubal pregnancy the corpus luteum was not in the ovary corresponding with the pregnancy in the tube but in the opposite one, which indicates that transperitoneal migration must have occurred. Leopold (1880) and Baur (1921) claim to have demonstrated external migration in the rabbit, and Corner (1915) originally held that this was of frequent occurrence in the pig (but see later). Our sheep material, as has already been stated, throws no light on this question.

Internal migration has been described, among carnivores, in the dog by Bischoff (1842, 1845), in the cat by Hill & Tribe (1924) and in the ferret (experimentally) by Hammond & Marshall (1930). We have found evidence for migration in a vixen in which there were three corpora lutea in the left ovary and one in the right; the foetuses (65 mm. C.R. length) were distributed two to each horn of the uterus.

In those carnivores (the European badger and possibly the American badger, certain martens and bears) which show the phenomenon of delayed implantation ('Entwicklungspause') one would expect to find transuterine migration, from one uterine horn to the other, more frequently than in the cat, dog and ferret, for in such cases there is more time available for 'spacing' of the eggs. Unfortunately, the data recorded in the literature on delayed implantation in carnivores does not enable us to determine if such migration occurs. Fischer (1931), however, in his work on delayed implantation in *Meles meles*, expresses the opinion that transuterine migration does occur in this carnivore.

Among the Cheiroptera there are at least five species with bicornuate uteri in which there is abundant evidence for uterine migration of ova and blastocysts (see Table 2). These species are of particular interest in that implantation is almost invariably in the right horn although ovulation may occur in either ovary. In *Glossophaga soricina* (Hamlett, 1934), with a uterus simplex, and in *Cynopterus marginatus* (Keibel, 1922), where the uterine horns open separately into the vagina, migration, on the data presented, does not appear to occur.

In the Insectivora, Brambell (1935, 1937) has shown conclusively that transuterine migration occurs in *Sorex araneus* and in *S. minutus*. In *Erinaceus europaeus*, Deanesly (1934) did not find it possible to count the corpora lutea, and hence correlation of them with the number and distribution of the embryos could not be achieved. She draws attention, however, to the fact that the embryos are distributed fairly evenly between the two horns. In our own small series of pregnant specimens of

Talpa europea we were also unable to count the corpora lutea with any feeling of certainty, and hence are unable to state if migration occurs in this insectivore.

In lower primates with bicornuate uteri few workers have given data on the distribution of the corpora lutea and the situation of the pregnancies. It is interesting to note, however, that Turner (1876) gave details of four pregnancies in *Lemur rufipes* which enable one to state that migration (transuterine or transperitoneal) must have occurred in one of them.

A few cases in the clinical literature suggest that transuterine migration may occur in the human in cases of interstitial ectopic pregnancy. The literature on this subject has been reviewed by Schlink (1924) who describes several well-authenticated cases. It is a theoretical possibility in cases of pregnancy in bicornuate uteri.

The data presented in this paper, for the sheep, and the literature on the development of the sheep, goat, cow, horse, pig, deer and *Procavia*, show quite conclusively that migration occurs frequently in the ungulates. This conclusion is supported by the experimental work of Warwick (1926) and Kelly (1928) who demonstrated transuterine migration in the sow when one uterine tube and the corresponding ovary had been removed. The frequency of migration is variable from species to species, being, apparently, most common in the pig and horse, less common in the sheep and goat, and rare in the cow. It is difficult to explain this variability, as the anatomical conditions are very similar in all of these species. It, presumably, can be attributed to species differences in the forces, whatever they may be, which cause the distribution of the eggs. Multiple pregnancy might be a contributory factor in the frequency of migration in the pig, but that it is not the only factor is shown by the apparently much greater frequency of migration in the horse as compared with the cow, although these two species are mainly monovular. Our results in the sheep, and those briefly recorded by Curson (1934), show that migration is common, in this ungulate, in double pregnancies resulting from two ovulations in a single ovary, but is rare in single pregnancies. It was found only once in all the sheep (95) on which we have data. Curson found it twice in thirty-six sheep.

Among the rodents, transperitoneal migration has been described by Leopold (1880) and by Baur (1921) in rabbits in which one ovary had been removed and the opposite uterine tube cut and tied. Baur found migration in five out of twelve experimental animals, and in one animal found as many as ten embryos in the uterine horn of the side on which the ovary had been removed. Asdell (1924) and Hammond (1935) also record that such migration occurs in the rabbit. Bischoff (1842) by comparison of corpora lutea and distribution of embryos in the rabbit found no evidence of migration. Parkes (1924), too, who, like Leopold and Baur, investigated the problem experimentally, found that not a single conception occurred in six rabbits in which one uterine tube and the contralateral ovary had been removed. Parkes suggested that Leopold's results might have been due to regeneration of the ovary on the side on which ovariectomy had been performed, but he admits that external migration may occasionally occur. Parkes does not refer to the work of Baur which not only verified Leopold's results but appears to have been carefully carried out and to have avoided the possibility of even the recanalization of the sectioned tube or of regeneration or incomplete removal of the ovary on the side in which pregnancy ensued. Further, Baur's counts of corpora lutea in the single remaining ovary agree completely with the number of pregnancies in the opposite uterine horn, and an acceptance of his results seems to indicate that not only can external migration occur in the rabbit, but that, under the conditions obtaining in his experiments, it is extremely common. In the rodents which possess a completely closed ovarian bursa (e.g. mouse, rat and field vole) transperitoneal migration is obviously impossible, and has been demonstrated to be so by Hanson & Boone (1924–5) and by Slonaker (1927) in the albino rat.

Transuterine migration is extremely unlikely in those rodents which possess uterine horns (better called right and left uteri) which open separately into the vagina. Leopold (1880) believed that his experiments on the rabbit demonstrated that both internal and external migration occurred. His results, however, were not checked by post-mortem examinations, and Baur's later experiments (1922) seem to demonstrate quite clearly that transuterine migration does not occur in the rabbit. Transuterine migration has been described by Bischoff (1853) and Bergmann & Leuckart (1857), on the basis of corpora lutea counts, in the guinea-pig, and in this species it is theoretically possible as there is a short common uterine segment. Kelly (1928), however, has shown that the uterine partition in the guinea-pig nearly reaches to the vagina and the experimental investigations of Kinney (1924), who performed unilateral ovariectomy in nineteen guinea-pigs and found no case of migration in subsequent pregnancies, appear to demonstrate that in spite of the presence of a short common segment, migration does not, in fact, occur in this species. This conclusion is supported by the descriptions of Reichert (1861), Squier (1932) and Nicol (1934). Transuterine migration, then, of the type found in Ungulata, Carnivora, Cheiroptera, Insectivora and at least one primate has not yet been demonstrated conclusively to occur in any of the rodents which have been studied with specific reference to such migration.

Probable mechanism

The question of the entry of the ovum into, and its migration through, the uterine tube has been discussed and the literature summarized by Westman (1926) and Grosser (1927); our material contributes no evidence on this problem. Our investigations involve the consideration of the mechanism of migration after the entry of the ovum into the uterus. In many mammals this involves the passage of the ovum or ova and their spacing, in multiovular species, either in one or both horns.

The problem is rather different in those species where the blastocyst remains relatively small and becomes early attached to, or actually penetrates, the endometrium, from those in which the chorionic sac becomes enormously enlarged and only a placenta of the epithelio-chorial or syndesmo-chorial type is developed. Even in species (e.g. rabbit) with a uterus duplex, in which transuterine migration is improbable, the problem of how the embryos become regularly scattered (spaced) and orientated in the uterus is unknown (see Mossman, 1937).

In most species at the time of the entry of the segmenting egg or morula no one part of the horn appears to be more favourable for attachment, nevertheless the embryos in most species become more or less regularly spaced; how this is achieved is unknown, but it obviously involves two main factors: (1) a mechanism for the transport of the ova, and (2) a mechanism which prevents the implantation of one ovum in close proximity to another.

Mossman (1937) suggests that the uterine mucosa in the immediate neighbourhood of an implanted ovum becomes refractory to the attachment of any other ovum. The migration of ova, blastocysts or chorionic sacs in those species (with bicornuate uteri) in which it can occur is obviously only a special instance of the general problem of the spacing of the embryos. The attempt at spacing, however, may not be completely successful; one embryo may remain in the common body of the bicornuate uterus, as was found in sheep nos. W 6 and W 7, and as has been recorded by Brambell (1935) in *Sorex araneus* and again (1937) in *S. minutus*. In Cheiroptera the migration is associated with a specialized receptive area in the right uterine horn.

There is no precise knowledge of the nature of the process involved in the migration of ova, blastocysts or chorionic sacs. It was, however, early recognized that ova and blastocysts have no inherent motility but that chorionic sacs may show slight 'creeping' growth. The forces must, therefore, be extrinsic to the ovum, and here there appear to be only two possibilities: activity of the uterine muscle or action of the ciliated endometrial epithelium. It would seem that the latter is unimportant, since cilia are not conspicuous features of the endometrial epithelia of most types. Lim & Chao (1927) have shown that the reversal of the middle portion of a uterine horn in the rabbit not only permits of pregnancy but also does not interfere with the distribution of the ova, showing that ciliary activity plays no essential role in the transport of ova within the uterus which must, therefore, depend wholly on uterine muscular contractions.

That the contractions of the uterine muscle must play the most significant role was suggested by Bischoff (1842) in his original account of internal migration. Volker (1922), in his *Normentafeln* on the development of the *Spermophilus*, describes the peristaltic uterine waves as 'seizing' the ova and carrying them onwards on the mesometrial border of the uterine lumen. In the interval between the peristaltic waves Volker believes that the ova can settle by gravity into the folds of the antimesometrial border of the uterine lumen, where they become implanted.

It is well established that uterine activity at first increases and then gradually diminishes following ovulation. Keye (1923) has described two types of spontaneous contractions in the isolated uterus of the sow; a major type upon which can be superimposed minor contractions. At the time of ovulation and during the passage of the ova along the uterine tubes the major contractions alone are present; the minor contractions begin with the functional development of the corpus luteum and increase until about the 13th day following ovulation. Keye believes that the minor contractions are concerned with the transport of ova through the uterine canal and their arrangement in a regular order for implantation. The full establishment of pregnancy alters the activity of the uterine musculature and causes a marked diminution of both types of contraction waves. Keye suggested that his physiological findings could be correlated with Corner's (1915) histological picture of the corpus luteum.

Lim & Chao (1927) confirmed the presence of peristaltic movements and also showed that antiperistaltic movements occurred. Their investigations were carried out on the rabbit in which transuterine migration does not occur, but their results can obviously be applied to explain the passage of blastocysts or even chorionic sacs (in those animals with non-deciduate placentae) into the opposite uterine horn from that which the segmented ovum or morula first entered. While these results of Keye and Lim & Chao seem to demonstrate a possible mechanism for the transuterine migration of the ovum the recent summary on the physiology of the uterus by Reynolds (1939) appears to show that the myometrium is quiescent long before ova reach the uterine lumen in mammals.

It is obvious that whatever forces are involved in transuterine migration they cannot be unidirectional, for the ova 'ascend' into the opposite horn.

This interpretation is supported by Lim & Chao's experiments on reversal of uterine segments in the rabbit. Reynolds suggests that 'perhaps the weak unco-ordinated motility which the uterus exhibits at this time is concerned in some way, but as yet no evidence is available to show whether or not this is so'. Reynolds concludes that, 'While it is clear that the mechanisms concerned in spacing' (and, we would add, in transuterine migration) 'are considerable, their nature is totally unknown at the present time'. We would, however, substitute *insufficiently* for *totally* in this statement, as the available physiological evidence clearly suggests that rhythmic peristaltic and antiperistaltic contractions of the uterine muscle may be among the main factors involved.

Significance of intra-uterine migration

Bischoff suggested that intra-uterine migration was a mechanism for balancing the number of embryos in each uterine horn when there has been a gross disparity in the number of ovulations from each ovary. Corner has supported this interpretation and advances the hypothesis that the phenomenon of transuterine (internal) migration has a practical utility in the pig in that it assures that the individual embryos of large litters should each find a fair share of space in the uterine cavity. This quasi-teleological explanation does not hold for species which are normally monovular. Thus transuterine migration is extremely rare in cows but common in mares. It has never been recorded for twins in cows but is, as we have shown, common in twins of sheep. These differences may be explained in part by subtle differences in the activity of the uterine musculature in the ungulates and in part on the fact that with only one or two pregnancies the need for spacing is not so urgent, especially as the chorionic sacs can later extend around into the vacant horn. Polyovulation is obviously a factor in the sheep where migration is frequent with double ovulations (from one ovary) but rare with single ovulations. On the other hand, in the horse, with single ovulations as the rule, intra-uterine migration is common. This is also true of many Cheiroptera. Polyovulation, then, is not the sole factor in the production of intra-uterine migration.

SUMMARY

1. Data are recorded which indicate that transuterine migration is of frequent occurrence in the sheep when double pregnancy results from two ovulations in a single ovary.

2. The histological appearances of the uterine cornua and body in the sheep are described.

3. The literature on transuterine migration is summarized and discussed.

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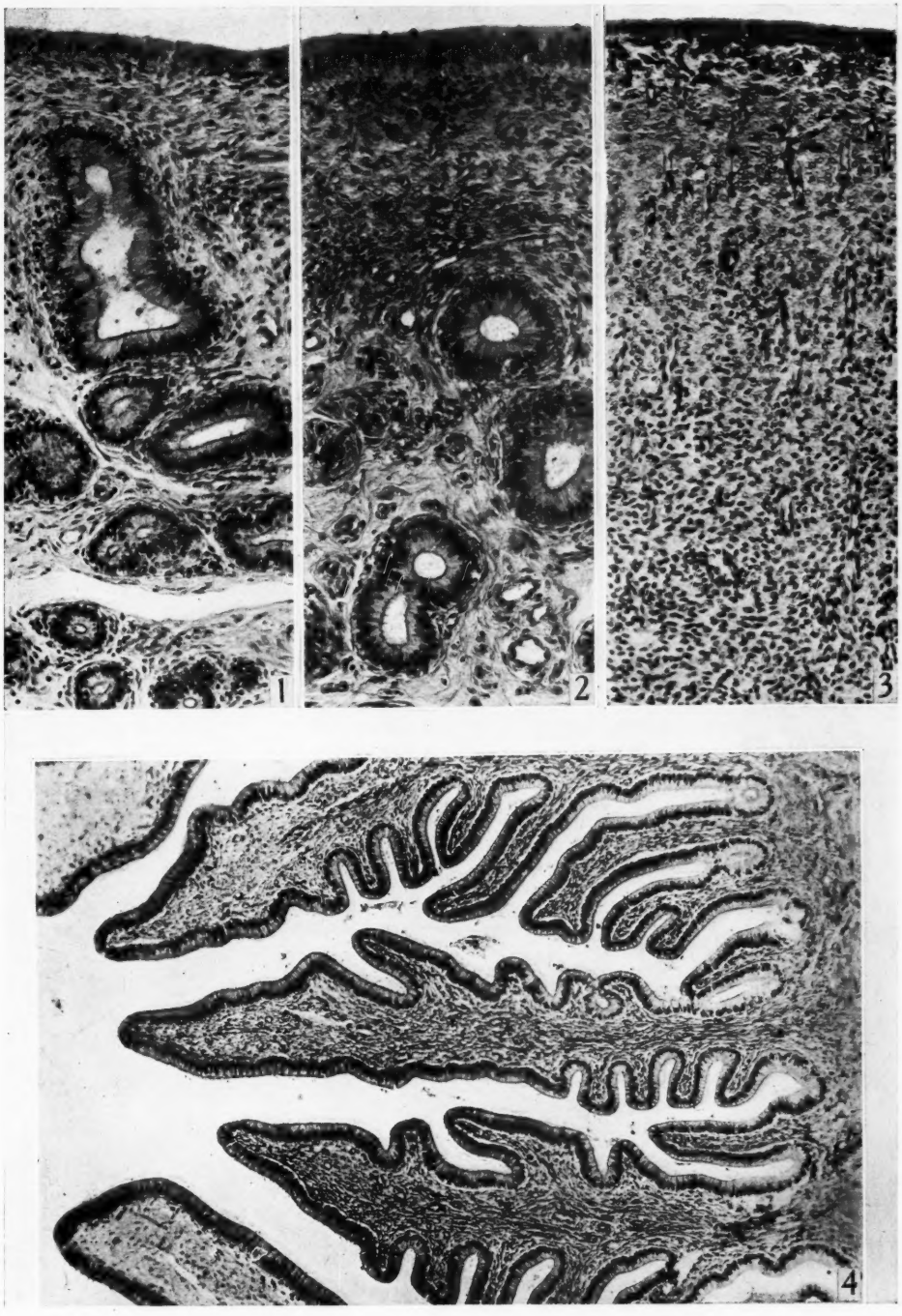
EXPLANATION OF PLATE

Fig. 1. Section through the glandular region of the endometrium of the uterine cornu. ×150.

Fig. 2. Section through the glandular region of the endometrium of the uterine body. ×150.

Fig. 3. Section through a caruncle (cotyledon) of the uterine body. A section through a caruncle of the horn has an identical appearance. The characteristic melanin-like pigment is seen immediately beneath the epithelium. ×150.

Fig. 4. Section of the cervical mucosa. ×150.



BOYD, HAMILTON AND HAMMOND—TRANSUTERINE MIGRATION OF THE OVUM

UNIV. OF MICH.

THE RE-INNERVATION OF MUSCLE AFTER VARIOUS PERIODS OF ATROPHY

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INTRODUCTION

Since a denervated muscle undergoes a progressive atrophy it might be expected that the process of its re-innervation would vary according to the time which elapses before new nerve fibres return. The existing accounts of regeneration of motor end-plates take no account of this possibility. Tello (1907) and Boeke (1916) studied muscles after severing the nerve at unspecified, but mostly rather small, distances (1–5 cm.) from its entry into the muscle. In some cases the stumps were sutured, in others (Tello) nerve fibres were left to cross a gap between the severed ends. From the information now available about the rate of nervous regeneration (Gutmann, Guttmann, Medawar & Young, 1942) it can be calculated that in the experiments of the above authors the first nerve fibres returned to the muscle after about 1–2 months. There is therefore no information about the re-innervation of muscles after longer periods of atrophy, such as are inevitable in man, even after immediate nerve suture, because of the great lengths of nerve to be regenerated.*

We have, therefore, attempted to answer the following questions: (1) What is the delay between the arrival of the tips of nerve fibres at a muscle and the onset of the power of transmission of impulses from one to the other? (2) Is this delay the same after short and long periods of muscle atrophy? (3) Do the end-plates remain intact throughout atrophy? (4) Is the process of re-innervation similar after short and long periods of atrophy? (5) If there is atrophy of end-plates, or failure to re-innervate them, can new plates be formed, even in the most atrophic muscle fibres?

The experiments are thus in effect an attempt to discover the extent to which the normal innervation of a muscle can be regenerated, and whether its capacity for recovery is progressively prejudiced as it atrophies.

METHODS

The method has been to study the state of innervation of muscles at various times after nerve lesions which had been made in such a way as to allow known periods to elapse before the return of nerve fibres to the muscle. For most of the study we have used the small peroneal muscles of the rabbit. These are all innervated by the peroneal division of the sciatic nerve which, being separated from the other divisions throughout the thigh region in the rabbit, can be interrupted at various distances from the muscle without affecting the functions of the tibial and sural divisions and hence without causing the production of sores on the heels. The muscles are most suitable for the investigation of the relationship between the structure of the end-organ and functional recovery, since three of them (mm. peronei II, III and IV) cause a movement of spreading of the toes which is easy to elicit reflexly by a sudden lowering of the suspended animal, and cannot be imitated by any other muscles (Gutmann *et al.* 1942; Gutmann, 1943). Although all the four peroneal muscles are of a convenient size for histological examination the three mentioned above are rather small for the preparation of frozen sections. M. peroneus I is ideal from this point of view, but has the disadvantage that its function (pronation of the foot) is not easy to elicit reflexly. Therefore

* Recently Naffziger, H. C. & Aird, R. B. (*Journ. Mt Sinai Hospital*, **9**, 1942) have examined muscles innervated after periods up to four months and found that regeneration was equally successful in all cases.

where accurate correlation of innervation and function was required we have made sections of peronei II–IV, but for studies of longer periods of denervation we have made histological examination of peroneus I, ignoring the slight discrepancy introduced by the fact that its time of recovery is a day or two in advance of that of the other peronei, whose movement was used as the actual index of recovery.

The study of long periods of denervation is made difficult by the impossibility of making a good union of stumps which have been held far apart—as is essential to avoid spontaneous union. Our method of overcoming this difficulty has been to make cross-unions of the tibial into the peroneal nerve (p. 29). Muscles were also examined from cases in which the tibial nerve had been kept without innervation for a long time and then re-innervated by union with the central stump of the peroneal nerve. At the second operation a similar cross-union was also made on the opposite side and it was then possible to compare re-innervation of gastrocnemius and plantaris muscles after a long delay and after primary suture (see p. 30). In both types of operation special precautions were taken to prevent spontaneous union of the stumps. A considerable stretch of the nerve (3–4 cm.) was resected and the central stump either stitched into the skin or else prevented from growing by injection into it of various inhibitors, especially formaldehyde (see Guttmann & Medawar, 1943). Examination of the pieces of the peripheral stump removed at the second operation showed that these precautions had in nearly all cases been successful, no medullated fibres being present. Occasionally a few small medullated fibres were found, and unmedullated fibres, invading the trunk from the periphery, were a regular feature (see Holmes & Young, 1942).

The histological method employed to demonstrate the nerve endings has been mostly that of Bielschowsky (see Weddell & Glees, 1941). The best preparations with this method show the finest branches of the nerve fibres, the nuclei, the cross-striations of the muscle and the sarcoplasm of the end-plate, with great clearness. Its weakness is that it is very difficult to obtain exactly the degree of staining which is desired. Different preparations vary in depth of stain, and particularly in its selectivity, in a manner which we have not been able fully to control. For a study of the re-innervation after increasing periods of atrophy it is especially important to have exactly comparable sections of each stage. Estimates of the success of innervation can only properly be made by counts, e.g. of number of innervated plates, and for these to be reliable comparable preparations are essential. By counterstaining with Ehrlich's haematoxylin we were able to make available some preparations which would otherwise not have shown such features as the number of end-plate nuclei. But the variation in the staining was a continual source of trouble and we cannot over-emphasize the importance, for any similar study, of using a standard and controllable technique. We are grateful to Mr W. Holmes for some excellent preparations made with his new silver-on-the-slide method (1943) which is not only readily controllable but allows of counterstaining with connective tissue stains.

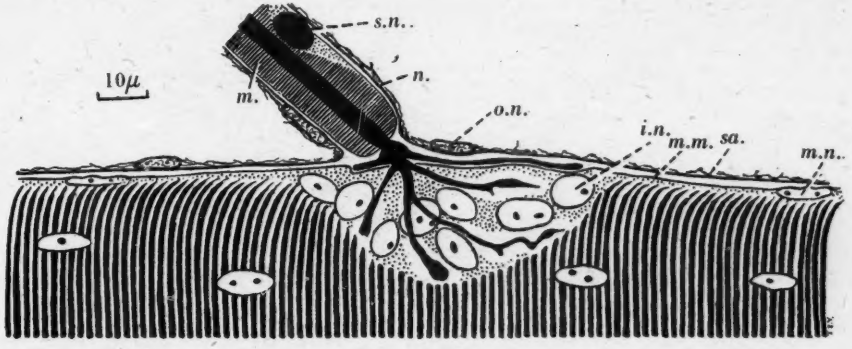
STRUCTURE OF NORMAL END-PLATES

A proper understanding of those aspects of the structure of end-plates which are significant for their normal functioning and regeneration involves an investigation of the nature of the membranes which surround the muscle fibre. The contractile portion of the muscle substance is surrounded by a very thin layer of unstriped 'sarcoplasm' which can only just be detected in normal muscle fibres but becomes more conspicuous during the atrophy of denervation (Text-fig. 1). In it lie the nuclei ('subsarcolemmal nuclei') of the muscle fibre. These are flattened to fit into the superficial layer of sarcoplasm, often having a thickness of as little as 1–3 μ when seen in lateral view, but appearing as elongated ovals of the order of $4 \times 12\,\mu$ in surface view. Each nucleus contains one, two or three large nucleoli. Its outer surface is often thrown into folds, whereas the inner one is smoothly rounded.

Unfortunately, there is much confusion as to the nature of the membranes outside this layer of sarcoplasm. Every text-book of histology states that a muscle fibre is surrounded by a 'sarcolemma', but there has been acute controversy on what structure should be called by this name (see e.g. Griesmann, 1913; Peterfi, 1913; Asai, 1914). Presumably there is a cell membrane at the surface of the sarcoplasm which is responsible for maintaining the difference in concentration between the outside and the inside of the fibre, separating a fluid rich in Na^+ and Cl^- outside from the K^+ space within. This membrane must be located at the outer edge of the thin band of sarcoplasm mentioned above, but there is no reason to suppose that it is a thick, visible membrane. Under favourable circumstances the edge of the sarcoplasm can be clearly seen, but presents no special membrane. The layer which is usually called sarcolemma is quite a thick membrane that lies more peripherally, and probably does not strictly belong to the muscle fibre but to the endomysium. There is no reason to suppose that this thicker layer has special permeability properties. We may therefore reasonably suppose that the muscle fibre is surrounded by two membranes: (1) a thin but physiologically important limiting membrane of the sarcoplasm, which may be called the muscle membrane or sarcoplasmatic mem-

brane, and (2) a thicker, visible layer, of the nature of connective tissue, the sarcolemma.

By means of a special staining method, Asai (1914) believed that he could reveal the very thin inner membrane as distinct from the outer connective tissue membrane. It is certainly not possible to do so with ordinary methods, and as Peterfi (1913, p. 23) says, 'that which we call sarcolemma in our preparations is in most cases nothing else but the thick connective tissue network.' Since the term sarcolemma has been, and still is, so often applied to the easily visible connective tissue membrane it would be pedantic and unwise to attempt to reserve the term for the 'true' or surface membrane of the muscle cell which however important, is rarely or never seen. For this latter membrane we shall use the term muscle or sarcoplasmatic membrane.



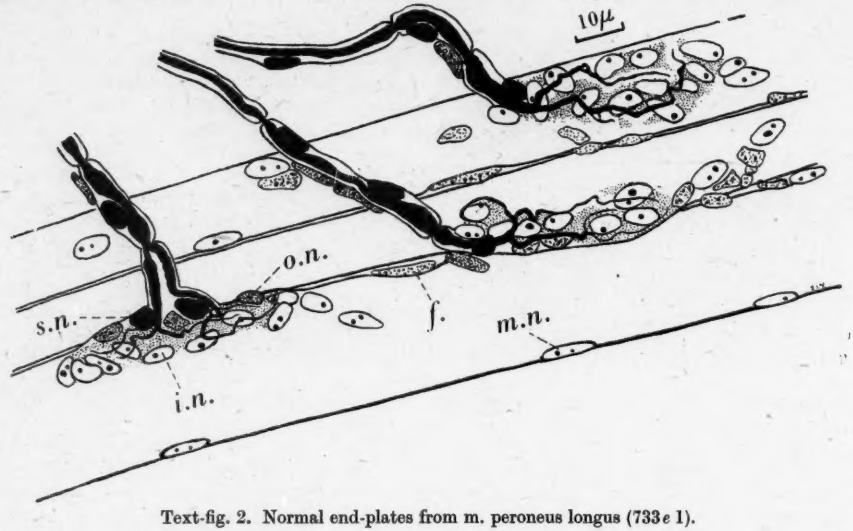
Text-fig. 1. Semi-diagrammatic view of a motor end-plate. The outlines are from a single plate, and the proportions are correct, but details have been adjusted to show the relations clearly. The main nerve fibre and sarcolemma are seen in optical section, but the rest of the plate covers the surface of the muscle fibre. Abbreviations for all text-figures: *cap.* capillary; *e.p.* end-plate; *e.e.p.* empty end-plate; *e.s.t.* empty Schwann tube; *f.* fibrocyte; *i.n.* inner end-plate nucleus; *m.* myelin; *m.m.* muscle (sarcoplasmatic) membrane; *m.n.* muscle (subsarcolemmal) nucleus; *n.* neurilemma; *n.e.p.* new end-plate; *o.n.* outer end-plate nucleus (fibrocyte); *p.* protoplasm of end-plate; *s.* fine fibre (? sensory); *s.a.* sarcolemma; *s.n.* Schwann nucleus; *s.t.* Schwann tube; *u.t.* ultra-terminal fibre.

The protoplasm of the end-plate is an expansion of the outer layer of the sarcoplasm, consisting of a mass of granular matter (Noël, 1927), sometimes raised into a considerable Doyère's hillock. Within the protoplasm lie the true nuclei of the end-plate (Sohlenkerne, of Kühne, 1864, Noyaux fondamentaux of Ranvier, 1878). These are large oval or rounded nuclei ($5 \times 12\,\mu$) containing one or two large, darkly staining nucleoli and a rather clear nucleoplasm (Pl. 1, figs. 1, 3, 8). A lateral view shows that they are compressed in the radial direction of the fibre, though they may reach a thickness of 3–4 μ and are thus less restricted in this direction than the ordinary muscle nuclei. Counts of fifty end-plates from normal peroneal muscles in adult rabbits showed an average of 8·1 of these nuclei, with numbers varying from 4 to 11. Towards the edges of the plates the nuclei are often smaller and less regular than at the centre, and transitions may be seen between these and the ordinary subsarcolemmal nuclei, from which, indeed, the end-plate nuclei do not greatly differ.

In the region where the incoming nerve fibre is connected with the end-plate there often lie other nuclei different from the above, about whose nature there has been some dispute. Ranvier divided them into noyaux vaginaux and noyaux de l'arborisation. In fact nuclei of Schwann cells or of fibrocytes may be present, but neither is a constant or essential component of the end-plate. The fibrocyte nuclei are the more usually present and may be called outer end-plate nuclei to distinguish them from the true or inner end-plate nuclei described above. These outer end-plate nuclei are of irregular oval outline, rather small (about $3 \times 5\,\mu$) and filled with fine granular 'chromatin' often staining darkly (Text-fig. 2 and Pls. 1 and 2, figs. 4, 10). In these respects they resemble the fibrocytes which lie in the endomysium between the muscle fibres and in the 'endoneurium' around the finer branches of the nerve fibres. Usually only one or two of them are present, sometimes none. This irregular distribution would be expected if they are the nuclei of the fibrous sheaths. In a lateral view of a plate they can readily be distinguished from the inner end-plate nuclei by the fact that they lie outside the branches of the nerve fibres (Pl. 1, figs. 2, 4). Other nuclei which may be

confused with those of the end-plate belong to the capillaries, which are abundant throughout the muscle, though not, so far as we have seen, especially so in the region of the end-plate (see however Noël, 1926).

The third type of nucleus which may be associated with the end-plates is that of the Schwann cells. These are easily recognized by their plump oval outline, dimensions of $5 \times 9\ \mu$ being typical. Usually they stain very dark black with Bielschowsky (Text-fig. 2). They are not strictly a component of the end-plate but may lie near it, especially when branching of the axon begins before the end-plate is reached so that the first branches are medullated (Pl. 1, figs. 6, 7).



Text-fig. 2. Normal end-plates from m. peroneus longus (733 *e* 1).

At the end-plate, the connective tissue sheath of the nerve fibre (endoneurium) joins that of the muscle fibre (sarcolemma, endomysium). The details of this union are not clear but may be conjectured to be as in Text-fig. 1. The nerve fibre runs down within a connective tissue tube, surrounded at least in part by a Schwann cell and by the neurilemma (see Holmes & Young, 1942) and passes into contact with the surface of the end-plate protoplasm. Branching may begin at some distance from the end-plate, so that several, in some cases as many as four, medullated branches may run side by side into the plate. Usually, however, the branching begins where the fibre first makes contact with the end-plate protoplasm, the fibre here dividing into two or more equal branches. Each portion then passes outwards towards the periphery of the plate branching sometimes two or three times, usually dichotomously (Pl. 1, figs. 1, 3, 5, 6, 7). Branching sometimes occurs in the proximity of a nucleus but this is fortuitous (see Hinsey, 1934). The two branches of each division are often subequal, but there may be very short collaterals which are difficult to distinguish from mere bulges. Each fibre tapers progressively towards the periphery, but irregular swellings occur. The final termination is usually in the form of a finely tapering point (Pl. 1, figs. 3–5), but there may be a slight bulb, a flattened disc or a ring (Pl. 1, figs. 2, 5). Out of forty-two consecutive endings examined at random, thirty-seven ended in fine tapers and only five were provided with a swelling at the extreme tip. Many authors have observed that some branches end in fine tapers (see Tello, 1905, 1907), but most have indicated that a terminal bulb or ring is the rule (Boeke, 1910, 1916). The appearance will of course depend largely on the technique, especially on the fixation and completeness of staining. In preparations made with gold chloride techniques the end branches usually appear swollen and varicose, perhaps as a result of the fixation employed. Moreover, Carey (1941) believes that there is a change in the shape of the endings, correlated with their functional activity. The final portion of the fibre is often flattened into a very thin sheet and hence is particularly liable to disruption in fixation. There is always the possibility that some portion at the end has been left unstained and indeed it is so thin that it often appears faint (Pl. 1, figs. 1, 3, 5). We are satisfied, however, that in most cases the branches shown represent the ends of the nerve fibres and that they may taper to fine points.

The relationship between the finest nerve

branches and the end-plate protoplasm has been the subject of much controversy. Boeke (1916) and others (see Hinsey, 1934) have maintained that there is a continuity of fine fibrils from the nerve fibre into a 'periterminal network' in the end-plate protoplasm. Wilkinson (1929, 1930) considers, however, that this is a misinterpretation, resulting from the artefacts of formol fixation. In considering the relationship it must always be kept in mind that the tissues have been fixed and stained, processes which cannot fail to have altered the appearance and, indeed, are designed to do so. The object of fixation and staining is not to discover the *appearance* of the living organs, which we know to be transparent. What the biologist does wish to know is the relationship between the nerve as a conductor, the end-plate as a conductor and, if possible, the muscle as a contractor. The fixation and staining of the preparation, and the looking at it, are experiments designed to clarify these relationships.

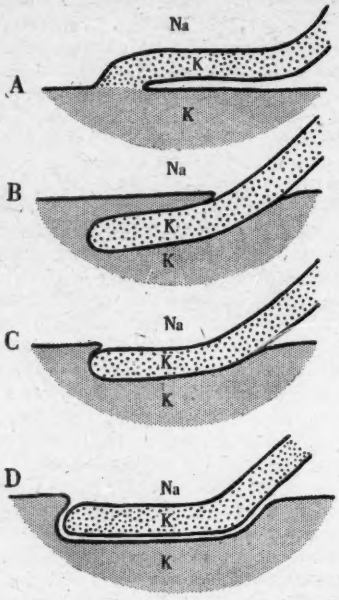
In making the investigation a clue is provided by the probability that, in relation to the conducting function of a nerve fibre, there must be at the outer edge of the axoplasm a boundary preventing the free passage of certain ions and allowing the maintenance of very different concentrations on its two sides, the inside being rich in K^+ and the outside in Na^+ and Cl^-. The most interesting aspect for investigation is, therefore, whether the axoplasm is at all points covered by a membrane or whether the appearances suggest that it becomes continuous with the end-plate protoplasm. The word 'continuous' here requires some attention. The axon is essentially a system bounded by a membrane whose presence, separating solutions of very different composition, makes conduction possible. Therefore the axoplasm is properly said to be *continuous* with some other plasm only when no membrane intervenes between them, so that the K^+ space inside the one is continuous with that inside the other. If a membrane or membranes intervene to prevent such a mixture the relationship is said to be one of *contact*.

The question of the exact relationship at the end of the finer branches is by no means easy to settle with existing techniques of fixation and staining. The finest branches often stain more feebly than the thicker ones. Moreover, being very thin, they are especially liable to disruption. Towards the tip the axoplasm sometimes appears to break into loose 'networks' (Pl. 1, figs. 1, 5), but these are such as might be expected to be produced during fixation of a very thin sheet of protein solution. When this happens to the very finest branches they become so dispersed that nothing is left to stain except a few scattered wisps or threads. These may give an appearance as if the fibre was breaking up into a number of excessively fine threads. It seems almost certain that this appearance is an artefact of fixation and that the living tip of the nerve fibre is a complete tapering cone or flattened disc (Pl. 1, figs. 2, 4). The division into fine wisps, even if it be not the result of fixation, is a rarity. Again, even if the finely divided threads be taken as a true representation of the living condition they still do not suggest a continuity with the end-plate protoplasm. The latter usually has a finely granular appearance after Bielschowsky staining, and this may sometimes give rise to a vague similarity to a network (Pl. 1, fig. 1). But this protoplasm is always more lightly stained than the nerve ending. Even where the latter appears to divide into very fine threads these yet terminate abruptly and do not pass over smoothly into the end-plate protoplasm. Indeed, there is often a slightly lighter portion of this protoplasm around the nerve fibre, along its whole length, as well as at its tip (Pl. 1, figs. 1, 8 and Text-fig. 2, cf. Wilkinson, 1930). This suggests that the finest branches *do not lie within the end-plate sarcoplasm at all, in the sense of being surrounded by it on all sides.* Boeke and others, from study mainly of surface views of end-plates, have considered the endings to be 'innerhalb des Sarcoplasmas der Sohlenplatte'. But in lateral view it can be seen that the branches lie either on the sarcoplasm surface or pressed into a slight groove (Pl. 1, figs. 2, 4). It is probable that the sarcoplasm is ionically a similar solution to the axoplasm, and it is not easy to see how an impulse could be conducted along the finer nerve branches if they were immersed in a medium of composition similar to their own interior.

There is therefore clear evidence that the axoplasm remains distinct from the end-plate protoplasm, in the sense that the membranes of the two remain complete at the point of contact. Moreover, there is a sodium space at least around part of the surface of the nerve fibre. Of the four conceivable arrangements indicated in Text-fig. 3 we may therefore conclude that either C or D is correct. This means that the relationship of muscle fibre to sarcoplasm is essentially similar in appearance to that of the members of a neuron-neuron synapse. Although it is clear that the insides of the nerve and muscle fibres are not continuous it remains uncertain whether the boundary between them should be considered as one membrane or two. The question is important for consideration not only of neuromyal conduction but also of regeneration. It may be that when the surface of the axoplasm touches that of the sarcoplasm reorientations take place in the surface layers of both, such that a single limiting layer is formed. The making of this contact might have important influences also on the 'trophic' condition of one or both partners.

It remains to consider the significant features of the shape and number of the terminal branches.

In a count of fifty end-plates taken at random from the peroneal muscles of adult rabbits the average number of branches within the end-plates was 5·8, with extremes of 2 and 11. This variation suggests that the number of branches is without special significance (see Hines, 1931; Carey, 1941), and the fact that most of them taper to fine tips, though a few are bulbous, suggests further that there is no special significance in the tip or final ending. The



Text-fig. 3. Diagrams of conceivable relations of nerve and muscle at an end-plate. A. Complete continuity of axoplasm and sarcoplasm. B. The nerve fibre penetrates the muscle membrane and runs within the sarcoplasm. C. The nerve indents the sarcoplasm and the nerve and muscle membranes fuse to make a single one. D. The nerve indents the sarcoplasm and the two membranes remain distinct, leaving a Na space all round the nerve. The actual relations are either as C or D.

significant portion of the end-plate is probably the whole stretch over which the nerve fibre comes in close proximity with the sarcoplasm of the sole plate. For this purpose the more proximal portions of the branches are presumably more effective, because larger, than the more distal. If this is true then the important portion of the ending is neither the tip, whether bulbous or not, nor any bulbs which may occur along the fibres. All these are 'accidents', and the effective contact is made by the whole stretch of unspecialized nerve fibre within the end-plate.

This hypothesis is confirmed and illustrated by the unusual plate shown in Pl. 1, fig. 8, in which apparently two branches, after passing separately over the end-plate protoplasm, have joined again. Except for two tiny lateral twigs the entire nervous part of the end-plate consists of a single circle. It is hardly likely that the two little twigs constitute the sole vehicle of transference of the impulse. Similar anastomosis of branches has been seen by Grabower (1902), Krebs (1905) and Boeke (1910), but was denied by Tello (1905).

The transfer of excitation by the 'collateral' action of a nerve fibre as it passes close to some other protoplasm has already been indicated in many other parts of the nervous system. Although no doubt special terminal synaptic buttons are often very significant, it is a mistake, at least in many cases, to ascribe special powers to the tip of a nerve fibre as such. This similarity of the synaptic surface to that of a simple nerve fibre emphasizes the probability that the process of transmission of excitation from one cell to another is not wholly different from that by which one portion of a fibre excites another portion of the same fibre.

RE-INNERVATION AFTER CRUSHING NERVE CLOSE TO THE MUSCLE

In a series of rabbits the peroneal nerve was crushed with forceps at a distance of about 20 mm. from the m. peroneus secundus, causing all the axons to undergo Wallerian degeneration. From previous work (Gutmann *et al.* 1942) we know that the latent period at the point of injury after this operation is 5 days, and that thereafter axon tips advance down the nerve at 4·4 mm./day. We do not know exactly how far fibres must travel within the muscle before reaching the end-plates. In two adult animals the point of nerve entry was found to be 6 mm. above a point half-way along the muscle. Adding a further 4 mm. for the course of the nerves within the muscle we may therefore suppose that new fibres arrive at the end-plates after $5+\frac{20+10}{4\cdot4}=12$ days. Accordingly, on the 12th and subsequent days one of the operated legs was examined, the nerve being exposed and stimulated 'faradically' with bipolar electrodes, the muscle being watched for contraction. After the experiment the muscle was removed for fixation and staining. In this way the results shown in Table 1 were obtained. The fourth column shows whether the reflex function of spreading the toes had reappeared at the time of examination and agrees with previous results (Gutmann *et al.* 1942; Gutmann, 1943) in showing that the function first returns about the 23rd day. Column 5 shows whether or not fibres were found in the intra-

muscular bundles, and confirms the expectation that the earliest arrive soon after the 12th day, though at first fibres are only present in a few tubes. Because of this it is difficult to be certain of the exact time at which axon tips first arrive at the end-plates (column 6). They were first seen in a few of the plates in a muscle examined on the 18th day after injury, and this was also the earliest muscle to show response on stimulation of the nerve (column 3). The delay between arrival of fibres near the plates and their entry into them cannot be greater than 5 or 6 days and is probably less. Further, in spite of minor inconsistencies such as would be expected, columns 3 and 6 agree very closely and show that as soon as fibres enter the end-plates they are able to cause the muscle to contract. Of course only a very small sample of all the plates in the muscle was examined, and moreover the visual test of 'indirect excitability' is also

Table 1. *Peroneal nerve crushed* 20 *mm. from its entry to m. peroneus II*

Latent period 5 days. Fibres arrive at plates after $5+\frac{20+10}{4\cdot4}=12$ days.

1	2	3	4	5	6	7
Muscle	Days after arrival of fibres	Indirect excitability	Spreading of toes	Fibres in bundles	Fibres in plates	Myelin
741*b*	0	0	0	0	0	0
712*b*	2	0	0	+	0	0
711*b*	4	0	0	+	0	0
712*e*	6	+	0	+	+	0
717*b*	7	0	0	+	0	0
711*f*	8	0	0	+	+	0
710*a*	9	+	0	+	+	0
695	11	+	+	Not studied		
688	14	+	+	Not studied		
710*h*	15	+	+	+	+	+
717*f*	16	+	+	+	+	+

far from perfect. But it is evident that after this short time of atrophy there cannot be more than one or two days' delay between the entry of a fibre into a plate and the development of its capacity to function.

The rapid return of function is perhaps not surprising, although the first fibres to enter the end-plates are very thin, since the plates themselves remain substantially unchanged after this short period of denervation. Pl. 2, figs. 9–11, show that after the disappearance of the nerve fibres from the end-plate the nuclei and protoplasm of the latter maintain their normal appearance. Counts (Table 2) of the number of nuclei in these denervated plates show no significant change in the inner nuclei of the end-plate proper. At this stage, at least, they have neither multiplied (and no division figures were seen) nor atrophied. On the other hand, the 'outer end-plate nuclei' appear to have doubled in number, and this would be expected if they are of the nature of fibroblasts which are at this time increasing in number throughout the muscle. However, the number of these outer nuclei varies so much that estimates are not very reliable. In later stages they again seem to be fewer; possibly, as Boeke suggested, they degenerate and disappear.

The Schwann nuclei of the finer branches within the muscle have also multiplied, but, as we have seen, these do not enter into the composition of the end-plate proper. There has been considerable difference of opinion as to the changes in the end-plate nuclei during degeneration (see Boeke, 1916). Some have described degeneration, others multi-

Table 2. *Counts of the numbers of branches and of nuclei in normal and regenerating end-plates*

The figures are the mean numbers, usually from counts of 15 plates. The figures in brackets show the extremes

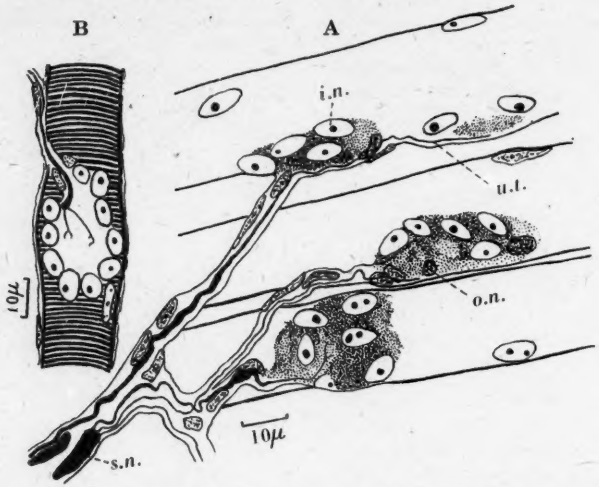
Muscle	Time after arrival of fibres days	Nerve branches	Inner nuclei	Outer nuclei
		Normal muscle		
Av. of 51 plates from 4 muscles ...		5·8 (2–11)	8·1 (4–13)	2·1
		After crushing nerve 20 mm. from muscle		
711*b*	4	0	6·8 (5–11)	4·4
712*f*	6	0·7 (0–3)	7·4 (4–13)	—
710*h*	15	3·2 (0–8)	8·0 (5–12)	4·6
695*a*	107	6·1 (2–12)	—	—
706*c*	188	6·8 (4–10)	10·0 (5–14)	—
		After crushing nerve 100 mm. from muscle		
727*a*	30	1·8 (0–3)	6·3 (3–10)	—
696*a*	64	4·7 (2–10)	7·7 (3–16)	—
702*e*	99	4·2 (1–8)	7·8 (3–13)	—
703*k*	169	8·3 (4–17)	11·2 (6–25)	3·0
704*g*	269	7·3 (2–14)	10·5 (5–18)	—
		After suture 48 mm. from muscle		
650*c*	65	2·4 (0–5)	8·4 (7–10)	—
762*f*	129	4·3 (2–11)	—	—
752*g*	126	3·9 (2–9)	8·3 (4–12)	—
671*f*	166	4·4 (3–6)	9·4 (6–13)	—
452*a*	281	3·7 (2–6)	6·0 (3–10)	—
569*a*	326	5·2 (3–9)	—	—

plication of the various types of nuclei, while Tello reported no change. Boeke observed the numerous small irregular nuclei at the centre of the empty plates, and thought them to be degenerating Telodendrienkerne, which he held to be specially differentiated nuclei. He noticed the variability in number of these nuclei and their disappearance in later stages. Although he never saw mitotic figures in the inner end-plate nuclei he held that they increased, perhaps amitotically, and as many as thirty-six were seen in a single plate. The only sign of multiplication of the inner nuclei in the present work has been a possible slight increase in the very late stages of re-innervation (p. 26).

At the period when new fibres are entering, or about to enter, the old end-plates, the sarcoplasm of the latter stains very darkly with the silver of Bielschowky's method. The plates therefore stand out clearly even when seen with low-power objectives (Pl. 2, fig. 11). This darkness of the plate appears to be a direct result of the approach of new fibres. In the case of muscles denervated for longer periods the plates were also found to stain when the new fibres approached them, although during the period of denervation they had remained unstained and very difficult to discern (p. 27).

A fine stream of axoplasm, returning along the Schwann tubes during the third week after crushing the nerve close to a muscle, is therefore led back directly to make contact with the end-plate sarcoplasm of a substantially unmodified plate (Pl. 2, fig. 13) which has only suffered from a slight reduction in size of muscle fibre and increase in connective tissue between the fibres.

At the entrance to the plates the stream is often, though not always, dammed up, producing a thickening (Pl. 2, fig. 13 and Text-fig. 4). It may be this obstruction which produces the delay between arrival of fibres and the first appearance of indirect excitability. Overcoming whatever produces the resistance the stream proceeds into contact with the end-plate protoplasm, at first as a very narrow strand. From this, as a result presumably of further obstructions met within the plate, little branches are thrown off, producing a ramified system over the surface of the end-plate sarcoplasm, between the inner and outer end-plate nuclei. It is not easy to be certain of the exact relationship of these early branches to the sarcoplasm, but there is every reason to believe that the nerve lies outside the sarcoplasmatic membrane, as in a normal plate. The tips of the branches end either as tapering threads or as slightly expanded, flattened bulbs (Pl. 2, figs. 14, 16). These sometimes have the appearance of rings, but this may be an artefact produced by the fixation of a thin plate of protoplasm. These formations are not spheres seen in optical section, for focusing shows that the dark margin is confined to a single plane. In other cases the appearance is not that of a simple ring but of a somewhat complicated network, this being another result of the fixation of small flattened sheets of protoplasm. Table 2 shows that even by the 27th day, that is to say after reflex function has returned, the number of branches is still much less than normal.



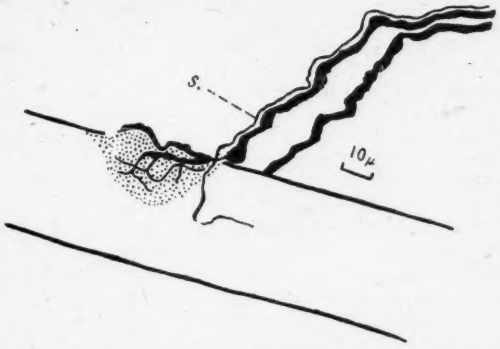
Text-fig. 4. Fibres entering end-plates. A. 18 days after crushing the nerve 20 mm. from the muscle (712*e* 1). B. 21 days after similar operation (710*a* 3).

The fact that, even though thin and few in number, the early branches are able to stimulate the muscles agrees with the conclusion reached on p. 20 that it is the presence of a nerve fibre at a plate which enables transmission to occur, rather than the development of any highly specialized end formation.

There is, however, one respect in which a definite abnormality appears even during this rapid reinnervation. At many plates the stream of axoplasm does not flow only into the end-plate. A portion of it escapes to make an 'ultraterminal' fibre,* running

* 'Ultraterminal fibre' has been used by some authors for the condition seen in normal end-plates in which a fibre arises from one of the branches within a plate and passes to some other ending (see Hinsey, 1934). The condition here described is not essentially different.

often for long distances between the muscle fibres (Pl. 2, figs. 15, 17, 18). In Text-fig. 4A there is a short ultraterminal fibre which has apparently stimulated the collection of some sarcoplasm making an addition to the old end-plate. These 'escaped' fibres will be further discussed later; here it may be noticed that they do not necessarily rapidly disappear. Indeed, in the series represented by Pl. 2, figs. 15, 17, 18 they appear to have become thickened and developed rather than to have atrophied. However, since in this quick re-innervation every muscle fibre rapidly receives a nerve fibre, presumably the ultraterminal fibres do not often go on to make new end-plates, if it be true that they are unable to do so with muscle fibres whose plates are already innervated. In fact no undoubted newly formed plates were found in this series, although they were abundant after longer denervation (p. 36).



Text-fig. 5. Nerve crushed 20 mm. from muscle 107 days previously. Two fibres run towards the end-plates, but the smaller (? sensory) fibre does not enter it. (695*a* 1.)

It is remarkable that from the earliest stages of the re-innervation each end-plate is almost invariably served by a single nerve fibre (Pl. 2, fig. 19), only in a very few cases have two fibres been seen passing to a plate. In most of such cases the two are branches of one axon, where branching begins before the plate is reached, an arrangement which is also quite frequent for normal plates. Even when the fibre can be followed for some distance it is often impossible to determine for certain whether or not the two fibres approaching an end-plate come from a single neuron. In the case shown in Pl. 2, fig. 21 two fibres run into a plate from apparently quite opposite directions. It is possible, but not certain, that in this case one muscle fibre is actually innervated from two neurons. In a few other cases in which two fibres not obviously branches of one axon approached a plate in a single tube, only one of them entered the plate, the other being either blocked or proceeding through the plate to end blindly in the endomysium (Text-fig. 5).

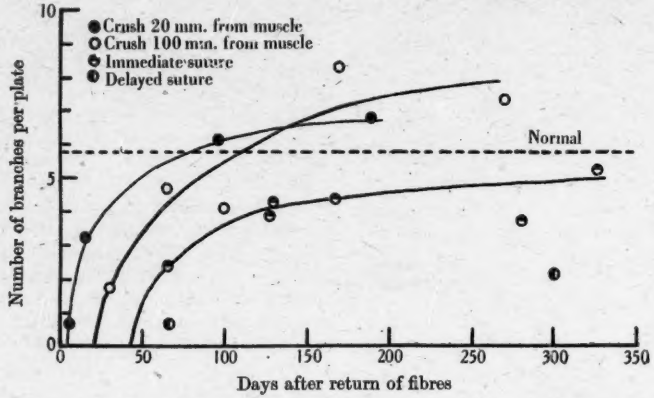
During outgrowth of a crushed axon many branches may at first be formed but they are mostly kept within the original endoneurial tube, and therefore led back to the old end-plate with which they were originally connected (see Young, 1942). Presumably only one of these branches proceeds for a considerable distance, growing at the expense of the others. Where more than one fibre runs within the endoneurial tube to the end-organ only one can enter. The other may eventually atrophy, but Text-fig. 5 shows that they may persist 3 months after operation.

When a nerve has been crushed in such a manner as to leave its endoneurium intact it is probably rare for a sensory fibre to grow down a motor channel or vice versa. However, some appearances were seen which suggest that sensory fibres have entered the muscle and instead of making contact with end-plates have proceeded to form elaborate plexuses (Pl. 2, fig. 20). The chief characteristics of these seem to be abundance of branching and the small diameter of the fibres. They were not seen in muscles examined 200 days after crushing, and it may be therefore that they are absorbed. However, since they are only occasionally present in any of the cases of this series their absence in one or two muscles may not be significant.

The great majority of the fibres returning to the muscle after this operation are therefore in their appropriate channels and proceed to reproduce end-plates which are very close to normal. Table 2 and Text-fig. 6 show that the number of branches within the end-plates returns to normal about 80 days after a nearby crush injury to the nerve. Muscles examined at about this time already show a condition approaching normality. The nerve fibres are approaching their original diameter. The branches within the plates are somewhat thicker than normal and an unusual proportion of them end in knobs, though many are tapered as in the majority of normal plates. At 107 days there are still some ultraterminal fibres, and indeed some at least of these have increased in diameter and become medullated (Pl. 2, fig. 18). In muscles examined 200 days after operation, however, the arrangement is very nearly normal (Pl. 2, fig. 22). The terminations within the plates are still abnormally thick and many of them are enlarged at the tip to form bulbs. However, there are also many which taper at the end. Ultraterminal fibres can still be found and may be medullated. Other persistent abnormalities are occasional terminal masses of axoplasm which appear to have gone astray and become encapsulated.

There are therefore many minor respects in which it can be recognized that a re-innervation has taken place, even as long as 200 days after denervation produced with the least possible damage, namely, by crushing the nerve close to a muscle. There is

little sign that any process of normalization by resorption of deviated fibres has taken place. However, the general pattern of re-innervation after such an operation approaches quite close to that originally present, since one fibre has flowed back down each old tube into the old end-plate (Pl. 7, fig. 73).



Text-fig. 6. Plot of the number of terminal branches found in end-plates during recovery from various types of nerve injury. Each point represents an average, usually of fifteen plates.

RE-INNERVATION AFTER CRUSHING NERVE AT A DISTANCE FROM THE MUSCLE

In a further series of animals the peroneal nerve was crushed at about 100 mm. from the m. peroneus longus. The reasons for using the peroneus longus in this and subsequent series are explained on p. 15. The distance from the point of entry of the nerve to the middle point of this muscle was found to be 12 mm. Adding 4 mm. as before, we may calculate that fibres would arrive back at the end-plates after about $5+\frac{100+16}{4\cdot4}=31$ days. Examination of indirect excitability and of the histological appearances was accordingly made at varying times after this period had elapsed, though no attempt was made to provide a complete detailed series.

The muscles examined after 35 and 42 days confirm that fibres have arrived in the nerve bundles in the muscle, though they are not yet to be found in the end-plates. These and subsequent muscles differ from those seen after a crush close to the muscle in that atrophy has proceeded noticeably further, reducing the volume of the muscle fibres, increasing the amount of connective tissue and making it difficult to recognize the end-plates. Nevertheless, there is no doubt that many if not all of the plates remain. They can often be seen before the nerve fibres have returned to them, though they become more easy to pick out when re-innervated. The counts of the numbers of nuclei in these plates show slightly smaller average figures than those found in normal and less atrophied muscles (Table 2), but the difference is barely significant. These numbers therefore prove definitely that some uninnervated plates persist for as long as 2 months, though it remains uncertain whether all do so.

The most noticeable feature of the re-innervation of these muscles as compared with those allowed to atrophy for only a short time after a low crush is the abundance of 'escaped' fibres, and their attempts to form endings. The longer period of atrophy makes a very big difference to the condition which the new fibres meet when they return. Presumably the 'escape' is a result of a change in the condition of the connective tissue (and neurilemmal?) tubes at the point of junction of nerve fibre and end-plate. The details about these membranes and their changes remain unknown, but it seems probable that as the muscle fibre shrinks and its fibrous investment thickens the connexion between the nerve fibre and the sarcoplasm at the end-plate is either broken, closed, or at least made less easily penetrable by new fibres (see p. 35 and Text-fig. 14). The result is that only a portion of the stream of axoplasm, or sometimes none of it at all, is directed back into the end-plate (Pl. 3, figs. 23–25). The rest passes on to make a new nerve fibre, running for a long distance between the muscle fibres. As a result the general pattern of nerves in the muscle is different from the normal and from that seen following crushing of the nerve close to the muscle (Pl. 7, fig. 74). Many of these long fibres are undoubtedly motor fibres which have failed to enter their end-plates, whereas the finer fibres (Pl. 3, fig. 26) may be sensory fibres which have entered motor pathways.

Such plexuses, running along the muscle fibre,

are never seen in a normal muscle, and they show that the nervous pattern produced during regeneration does not necessarily follow exactly the old lines. The Schwann tubes in the nerve trunks and plexuses are very valuable guides leading along new fibres towards their end-organs (Holmes & Young, 1942; Glees, 1943), but they do not inevitably ensure that connexion will be made with the latter.

The new fibres are laid down in the connective tissue spaces between the muscle fibres, which evidently provide admirable opportunities for spinning new threads. This is indeed a good example of the way in which new nerve fibres can be laid down, along suitable tracks, in the absence of pre-existing Schwannian or other pathways. They soon come to be accompanied by fibroblasts and by Schwann cells, which must have migrated on to them. In later stages such new fibres can undoubtedly become medullated.

Many of these escaped fibres can be seen to terminate in the intermuscular connective tissue (Pl. 3, fig. 31). Presumably the stream of axoplasm becomes blocked (though the ending is often of a fine tapering form) and fails to make contact with a muscle fibre. But it is also possible for such wandering streams to penetrate the membranes covering the muscle fibres and to make a new plate. This can be conclusively proved by such cases as those shown in Pl. 3, figs. 28 and 29. Here the very simple plates, with their single endings, are found on the end of fibres which, farther back, can be seen to have escaped from an old end-plate. Such an arrangement of end-plates in series is never seen in normal muscles, and the endings shown are certainly, as their appearance suggests, new end-plates. Where a nerve fibre makes direct contact with the sarcoplasm it produces a differentiation of that substance into the material characteristic of the end-plate. This is not surprising, since we know that the same happens in development (Boeke, 1910), but it is interesting to obtain definite proof of this organizing power of the nerve fibre in the adult. The changes involved are not, perhaps, very extensive, mainly the collection of a little mass of sarcoplasm and a few muscle nuclei.

Other 'attempts' at the formation of endings are seen (Pl. 3, fig. 30), but it is only rarely possible to be sure of the exact relationship of the nerve fibre to the sarcoplasm.

In spite of these ultraterminal formations the great majority of the newly innervated plates seen after a crush 100 mm. from the muscle are probably persistent plates. But the new branches are not formed within them in quite the same manner as after a closer lesion. The greater atrophy of the muscle fibre appears to have compressed the end-plate protoplasm and the space above it, so that the new stream of axoplasm enters the plate only with difficulty and often makes at first large lumps (Pl. 3, figs. 23, 27, 32). Finer branches and bulbs such as those seen after a shorter time of atrophy may, however, also occur (Pl. 3, figs. 25, 27). But in general the impression is that the stream of axoplasm meets with a considerable resistance which delays its penetration. The fine fibres can be seen, in favourable cases, to indent the sarcoplasm (Pl. 3, fig. 27), as at normal endings. The 'ultra-terminal' or 'escaped' portion of the axoplasmic stream is usually so prominent that the portion innervating the original plate itself is now more in the nature of a collateral (Pl. 3, fig. 23). However, in spite of the difficulty of innervation suggested by these various formations, it is clear that the new fibres have succeeded in making contact with the muscle fibres largely by means of the old plates. Correspondingly, these muscles acquire the power to function at a time not very long after the arrival of new fibres. The interval appears from Table 3 to be not more than 12 days, assuming that fibres arrive on the 31st day. This is a little longer than after the very short period of atrophy imposed by a crush close to the muscle, but evidently there is no major delay before the onset of indirect excitability. The data of Gutmann *et al.* (1942) and Gutmann (1943) show that return of reflex function after such a lesion occurs about the 53rd day, that is to say with a delay after arrival of fibres of about 22 days as compared with 11 days after a low crush. Part of this extra delay may be due to the greater difficulty with which the fibres enter the plates in the more atrophied muscle, but it must not be

Table 3. *Presence or absence of indirect excitability in m. peroneus longus*

A. After crushing the peroneal nerve 100 mm. from the muscle. Fibres return to the end-plates after $5+\frac{100+15}{4{\cdot}4}=31$ days. Recovery of spreading of toes occurs about the 53rd day

Animal	Days after operation	Indirect excitability
778	35	0
779	42	0
561	43	+
727	61	+

B. After suture 48 mm. from the muscle. Fibres return after $7+\frac{48+15}{3{\cdot}5}=25$ days. Recovery of spreading of toes occurs about the 50th day.

Animal	Days after operation	Indirect excitability
779	35	0
778	42	0
685	48	+
857	52	0
753	55	+

forgotten that where a greater length of nerve has to be regenerated there may be increased delay between arrival of fibres and recovery because of the need for greater medullation, and other factors may also increase the delay (see Gutmann *et al.* 1942).

Function returns in spite of the abnormal shapes of the fibres in most of the endings, and this is further evidence that it is the presence of a fibre in the sarcoplasm which makes excitation possible. Moreover, as after crushes close to the muscle, not only the shape but also the number of endings in these early plates is grossly abnormal (Table 2).

The subsequent process of normalization of the end-plates is shown in Table 2, Text-fig. 6 and Pl. 3, figs. 33–37. It takes a long time. In muscles examined 3 and 4 months after the interruption the number of endings in the plates is still abnormally small (Pl. 3, fig. 33), and they are often unduly thick. Some of the plates can now be seen to be 'terminal', but many still have 'ultraterminal' fibres attached to them, and such escaped fibres are still abundant running between the muscle fibres. However, some of the excess of nerve fibres may have been reduced, and the plexus among the muscle fibres seems less complex than in the muscles examined during the early stages of re-innervation. The newly formed end-plates can still be recognized by their very small number of end-branches and nuclei, as well as by their arrangement relative to the larger nerve trunks in the muscle and to other plates. However, some of the plates at this time have a structure indistinguishable from normal.

The plates seen in the muscle examined 200 days after such a crush contained a significant excess of end-branches and nuclei (Table 2), and the branches and endings were often unusually stout (Pl. 3, figs. 34–36). Some (fig. 36) can be seen to be definite loops (see p. 22), others flattened plates. Perhaps as the muscle fibre increases in volume after its atrophy the sarcoplasm of the end-plate also spreads out and allows the formation of new branches. There is no evidence as to how the increase in nuclei takes place, whether by division or the incorporation of new subsarcolemmal nuclei into the plate.

In this muscle there was very little sign of long 'ultraterminal' fibres running between the muscle fibres. In fact, the general pattern of the innervation is now more close to normal, with most of the plates terminal. This condition may be contrasted with that found at a similar time after suture, when an excess both of large and small fibres is still present in the muscle (p. 29).

A muscle taken 300 days after crushing the nerve at this level showed no signs of nerve fibres running for long distances between the muscle fibres, and only few of the plates were collateral. Presumably, therefore, the excess fibres are gradually absorbed, though the process must be a very slow one. Many of the plates could not be distinguished from normal at this time, others still showed signs of the injury in the persistence of various irregular lobes and masses (Pl. 3, fig. 37). Many of the branches within the plates now end in a taper, or with very fine terminal expansions, but rather more of them end in blunt knobs than is normally the case. The number of branches and nuclei within the plates is rather high, and in many plates the mode of branching and course of the fibres are less regular than normal.

Thus, 300 days after a crush at 100 mm. from the muscle the innervation, though very abundant, is still not quite normal in appearance. The great excess of fibres which appears in the early stages after this operation has certainly become somewhat reduced. The processes of adjustment which have to proceed after arrival of the fibres at the muscle appear to be (1) the entry of branches into old plates, (2) the formation of new plates, (3) the elimination of deviated fibres which have missed their way. After a crush close to the muscle the process of regeneration consists mainly of process (1). After a more distant crush (1) proceeds with greater difficulty and (2), which rarely or never occurs after a low crush, is found. Since so many fibres fail to flow straight back into end-plates, the third process, removal of excess fibres, is much more important after a distant than after a close lesion.

RE-INNERVATION AFTER PRIMARY SUTURE

After severance and immediate suture of a nerve all sensory and motor functions recover later, more slowly and less perfectly than if the nerve has simply been crushed. We have investigated the recovery of the muscles during re-innervation after denervation for various periods. The usual level at which sutures were made was 48 mm. from the m. peroneus longus, and from the data of Gutmann *et al.* (1942) we may calculate that when primary suture is made immediately after severance fibres should begin to arrive back at the end-plates after $7+\frac{63}{3{\cdot}45}=25$ days of atrophy. Table 3 shows that there is a delay of at least 17 days between the arrival of fibres and the onset of indirect excitability, as compared with a minimum of 6 days after a crush close to the muscle and 12 days after a more distant crush. After sutures at the level we are considering the function of spreading the toes was found by Gutmann *et al.* (1942) to return after 54–70 days. Later observations have shown recoveries as early as 47 days, and 50 days is probably a reasonable figure for a good suture at this level. The longer times in earlier experiments were the

result of imperfect unions, which are liable to result from the tension on the nerve at this level. There is therefore a delay after a good primary suture at this level of about 25 days between the return of the first fibres to the end-plates and the appearance of reflex function. The comparable delay after crushing the nerve close to the muscle is 11 days and after a more distant crush 22 days.

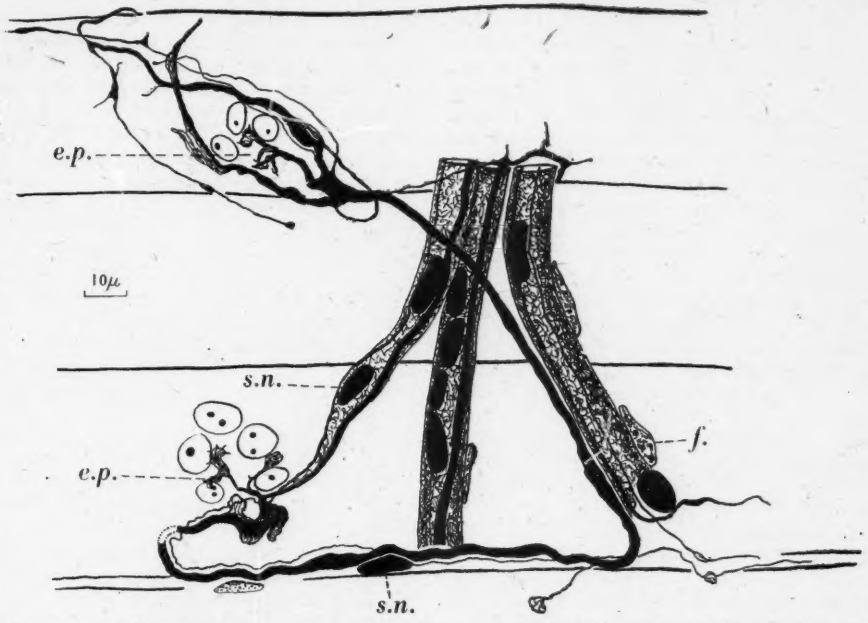
The muscle examined 35 days after operation, that is to say 10 days after the calculated time of return of the first fibres, shows quite thick fibres in some of the nerve bundles (Pl. 4, fig. 44). But only a few tubes in each bundle contain fibres, in marked contrast with the condition seen in the bundles at a similar period after a crush, where every tube contains fibres (Pl. 4, fig. 43).

Some of the fibres have already penetrated into end-plates and produced several branches there. Such re-innervated plates stain darkly and in every way resemble the plates seen during re-innervation after crushing. 'Escaped' fibres are common, and there is some formation of longitudinal plexuses of fibres, though perhaps less than occurs during re-innervation after delayed suture.

The fact that re-innervation has proceeded well in this muscle may be connected with the small amount of atrophy which has been produced. There is considerable variation between individual rabbits in this respect. The animal examined 42 days after operation showed a much more pronounced atrophy and very little re-innervation of end-plates, although fibres are abundant in the nerve bundles in the muscle.

The animal examined at 48 days (23 days after arrival of fibres) shows less atrophy than the last, but here again, although fibres are present in the muscle, no innervated end-plates were seen.

After one month of atrophy and one of re-innervation the muscle contained many plates in process of re-innervation and these appear dark.



Text-fig. 7. Nerve fibres 30 days after their arrival in a muscle after suture. Three Schwann tubes are shown. That on the left contains a large and a small fibre, but the latter (? sensory) runs past the end-plate. The motor fibre sends branches into the plate and then runs as an ultraterminal fibre along the muscle and back up along another tube to end in complex branches on another muscle fibre (753*d* 9).

It will be remembered that after crushing the nerve close to the muscle the plates also appeared conspicuously dark during early re-innervation, and this dark staining therefore appears to be a general feature during the stage at which the fibres first reach the plates.

In most cases only a single fibre runs to each end-plate. This arrangement is therefore not a result only of crushing, where each fibre remains within its own tube. Presumably of the many fibres which enter each tube of the peripheral stump below the suture (Holmes & Young, 1942) only one usually succeeds in reaching as far as the muscle. However, in some cases, as shown in Text-fig. 7, more than one

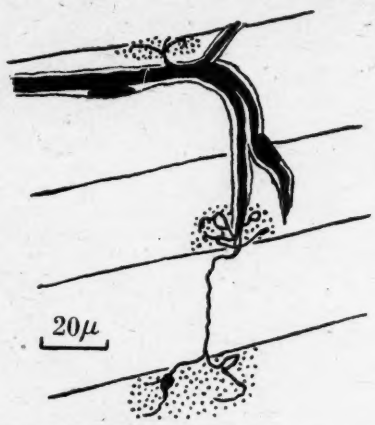
fibre approaches a single plate. Since it is almost universal in normal muscles for each plate to be approached only by a single fibre, presumably in the case shown in Text-fig. 7 the two are in one tube. Yet only one of them sends processes in to the end-plate, the other passing by and becoming lost in the endomysium. In other cases also, where more than one fibre was seen approaching an end-plate, only one succeeded in entering it; the other being blocked and, turning back, forming a small end-bulb facing away from the end-plate (Pl. 4, fig. 39).

Many of the re-innervated plates closely resemble those found after crushing, some being quite well-formed terminal plates. Others have ultraterminal fibres of the familiar form (Text-fig. 7). There must also be many cases in which sensory fibres, entering false pathways, arrive at motor end-plates. Gutmann (unpublished) finds that such fibres cannot make anatomical or functional connexion with the muscles. After suture of the sural (sensory) into the peroneal nerve stimulation produces no contraction of the muscles supplied by the latter. Presumably when such fibres arrive at the end-plate they 'escape' and produce some of the plexus arrangements which are seen in the muscle. These plexuses are a constant feature of muscles re-innervated after suture and are more conspicuous at all stages than after crushing the nerve. They appear specially clearly in the muscles examined about 100 days after suture. In these there are many fine fibres running along between the muscle fibres, but often also crossing and even wrapping around them (Pl. 4, fig. 42). On account of the changes in plane it is very difficult to photograph these fibres. It is not possible in any one case to be sure that they are sensory, but it seems likely that many of them are so.

The new motor fibres within the plates are often thickened (Text-fig. 7 and Pl. 4, fig. 38) as if they had met with resistance on entering the plates, and indeed the whole fibre for some distance behind the plate may be swollen. 'Escape' of part of the axoplasmic flow is almost the rule, so that nearly all of the end-plates are collateral. The thickening of the fibres within the plates leads to the production of more knobs and end-bulbs than are found in normal plates or during regeneration after shorter periods of atrophy. Presumably the outflowing stream of axoplasm finds difficulty in penetrating into the end-plate because of the shrinkage of the muscle fibre and constriction of the end of the Schwann tube. In Text-fig. 7 it can be seen that the stream of axoplasm emerges from the end of the tube and makes a large irregular mass, part of which proceeds into the old plate while the rest forms an ultraterminal fibre.

Because of the bizarre forms of most of the plates at this stage it is not possible to make a count of the number of endings which is exactly comparable with those previously given. But from the few figures which could be obtained and are shown in Table 2 and Text-fig. 6 it is clear that the number at this time is far below normal and presumably many of the plates have none at all, but these empty ones are so difficult to detect in the atrophied muscle that it is not possible to obtain a satisfactory idea of the proportion re-innervated.

In muscles examined 90 and 99 days after suture, that is to say after 1 month of atrophy and 2 months of re-innervation, there has been a considerable normalization of some of the plates, and counts of endings, which are now somewhat easier to make, show that there has been further branching within the plates. Many, however, still remain very simple in form (Pl. 4, figs. 41, 45). One nerve fibre may innervate several plates 'in series', some of these



Text-fig. 8. Single fibre innervating several end-plates in series, 2 months after return of fibres to the muscle after suture (708*c* 5).

being new ones (Text-fig. 8). A great many 'escaped' fibres can still be seen running for long distances between the muscle fibres, and some of them are medullated. Some of these fibres end in tapering points or knobs and others give off collaterals (Pl. 4, fig. 46), in fact, the stream takes many different directions. Various types of plexus, often of fine fibres, lying across the muscle fibres are also seen, and some of these are presumably sensory.

Even 4 months after fibres have returned to the muscle the number of endings in the plates is still very far below normal and the plates still show an abnormal collateral position (Pl. 4, fig. 47). However, many plates are also terminal, and it is clear from the general pattern of innervation that much of the re-innervation has been of the old plates, lying in their usual distribution close to the nerve

bundle so that the general pattern may be not grossly dissimilar to that seen in normal muscles (Pl. 7, figs. 75–78).

Even after 6 months the number of branches in the end-plates was still found to be abnormally low, both in a muscle whose nerve had been sutured and one where a nerve autograft had been made. The plates are still nearly all recognizably abnormal. Many have somewhat thickened fibres (Pl. 4, fig. 51). In other cases the plates remain very simple, with only one or two terminal branches (Pl. 4, figs. 49, 50). However, in many plates there are numerous terminals ending as fine tapering threads, much as in normal plates (Pl. 4, fig. 48). Many of the plates are terminal, although ultraterminal 'escaped' fibres are still abundant in the muscle. Some of these end blindly in the endomysium, but others go on to form plates which must be new since the innervation of two muscle fibres in series is never seen in normal muscle. There are also still many fine non-medullated and small medullated fibres forming a plexus between the muscle fibres, and these are probably sensory (see p. 28). In this respect the muscles examined 200 days after suture contrast strongly with those whose nerve had been crushed and which at this stage showed a nearly normal pattern of innervation. If the finer fibres in the muscles after suture are sensory it is clear that there is no rapid reduction of these aberrant connexions.

Further confirmation of the persistence of such plexuses was found in a gastrocnemius muscle examined 10 months after cross-suture of the peroneal into the tibial nerve (452*a*). The conditions after such cross-unions are of course somewhat abnormal, in that many of the connexions made are likely to be non-functional (see p. 30). It might perhaps, therefore, be expected that the plexuses of escaped and sensory fibres would be resorbed especially readily. Pl. 4, fig. 52 shows, however, that some of them at least are still well developed 10 months after suture, and indeed seem to contain thicker fibres than in the earlier stages. Comparison of the innervation of this gastrocnemius muscle with one which was examined only 3 months after suture (426*b*) showed that if anything there had been an increase with time in the number, and certainly in the thickness, of fibres in the plexuses.

The end-plates in the muscle examined 10 months after suture are well developed but mostly abnormal in the small number, shortness and sometimes thickness of their branches. Counts of nineteen plates showed an average of only 3·7 terminal branches per plate, with extremes of 2 and 6; the average number of nuclei was also low (6·0). Some of these plates are so simple that they are probably new ones, but it is difficult at this stage to identify them with certainty.

At such a long period after suture there should have been time for all of the muscle fibres to have recovered, had they been quickly re-innervated. There are, however, some areas in which the muscle fibres, though not grossly atrophied, are still small. It seems that they must have continued to atrophy for a longer time than the 25 days before return of fibres to the muscle, presumably because their connexion with appropriate motor nerve fibres was long delayed.

One m. peroneus (569*a*) was examined exactly a year after a suture in which the union was shown by sectioning to be a poor one, with a gap of approximately 2 mm. between the stumps. The general condition shows little change from the muscle at 200 days. The number of terminations is still somewhat below normal in each plate, but most of them taper or end as small knobs. Both terminal and collateral plates (Pl. 7, fig. 82) are seen, and the 'escaped' fibres proceeding from the latter may become thick and heavily medullated fibres, though in other cases they remain very thin. The general impression even after this long period of recovery is that the innervation is abnormal in that the number of nerve fibres is large, although there are abnormally few innervated plates. Normalization both by development of innervated plates and reduction of excess of fibres must be, at best, a very slow process.

RE-INNERVATION AFTER DELAYED SUTURE

The methods by which muscles were kept for long periods without innervation have been described on p. 16. The material available forms two series: (A) those in which a section was removed from the peroneal nerve and then at a second operation the peripheral stump re-innervated by union with the tibial central stump (Table 4); (B) those with the converse procedure, section of the tibial and its re-innervation from the peroneal (Table 5). In series A the intervals between the two operations were 1, 2, 4, 6 and 8 months. Since the suture was performed about 40 mm. from m. peroneus longus, we may calculate that fibres would arrive back at the muscle about $7+\frac{40+16}{3\cdot5}=23$ days after the operation. It is known that fibres may grow through a peripheral stump degenerated for a long time as fast as through one which has been freshly sectioned (Holmes & Young, 1942). However, there are various factors which are likely to delay the arrival of fibres under these conditions (for instance, the union is probably less successfully made). And we may therefore suppose that return of fibres took place not earlier than one month after suture, and therefore that in the cases mentioned above the muscles had undergone atrophy for 2, 3, 5, 7 and 9 months.

All the animals in this series were killed 90 days after the second operation, that is to say, about two months after return of fibres to the muscles. They can therefore be compared with the animals which were examined 90 days after a primary suture (p. 28). However, since in the present cases a cross-union of tibial into peroneal is involved, three animals were also examined in which a primary cross-suture of this type had been made 90 days previously. No difference could be detected between the structure of the muscles innervated by their own (peroneal) and by tibial fibres. Both of these are mixed nerves, and the tibial being larger than the peroneal can amply supply it. A small proportion of the fibres in the tibial runs to the m. abductor hallucis which operates the spreading of the first toe, i.e. it acts together with the small peroneal muscles which spread the other toes (p. 15). Perhaps because of the presence of these fibres some spreading of toes II–IV reappears after cross-union of tibial into peroneal, though it never reaches the extent which is found after direct peroneal suture. As has already been reported (Gutmann, 1943), the time of reappearance of this function becomes considerably delayed after the longer times of atrophy found in the present series (see Table 4). But all of the animals had shown some recovery before they were killed 90 days after the second operation, though in the case of the animals with the longer time of atrophy movement had only just appeared.

Table 4. *Comparison of delay, after various operations, between return of fibres to end-plates in peroneal muscles and onset of indirect excitability and function. The results for the first three types of operation are averages*

Operation	Animal	Period of atrophy months	Delay between return of fibres and of indirect excitability days	Delay between return of fibres and onset of spreading days
Crush 20 mm. from muscle	—	½	6	11
Crush 100 mm. from muscle	—	1	12	22
Primary suture 48 mm. from muscle	—	1	17	25
Delayed suture 48 mm. from muscle	642*d*	2	—	32
	650*e*	3	—	49
	680*a*	5	—	35
	595*a*	7	—	35
	597*a*	7	—	30
	581*a*	9	—	56

In the second series of animals, in which the tibial nerve was innervated after various periods of atrophy, from a peroneal central stump, no functional recovery appeared. The peroneal is smaller than the tibial, but this will not prevent most of the tubes in the peripheral stump being reoccupied by branching of fibres of the central stump. However, the only reflex movement which the nerves operate together is spreading of the first toe, and apparently the small m. abductor hallucis is too weak, after crossed re-innervation, to produce a visible movement. However, all the muscles examined gave powerful contraction when the nerve was stimulated at the time of biopsy. There is much evidence to show that motor fibres are able to form effective connexions with muscle fibres of functions very different from those which they normally operate. Though little is known of the later history of the function and structure of these irregular connexions there is no special reason to suppose that in the early stage they will differ in appearance from those found during direct re-innervation. However, it cannot be assumed that functional factors have no influence on the structural re-innervation, and indeed it is highly likely that in the later stages they will do so. It can hardly be supposed that the recovery of a muscle fibre and of its end-plate follows the same course whether or not it is performing effective movements.

Table 5. *Cases in which the peroneal nerve was sutured to the tibial after various periods of atrophy and gastrocnemius and plantaris muscles then examined*

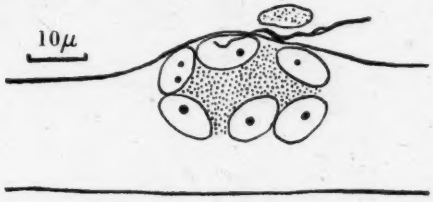
Muscle	Time between severance of nerve and suture months	Total time of atrophy before return of fibres months	Time between arrival of fibres and fixation of muscle months
P11	10	11	2
P10	12	13	2½
P12	12	13	2½
426	16	17	3
452	16	17	10

However, our present purpose is to discover the effect of atrophy on re-innervation and to obtain muscles for comparison with those cross-innervated after long delay. We performed, at the second operation, control *primary* cross-sutures of the peroneal into the tibial on the opposite leg. The gastrocnemius and plantaris muscles from this control series appeared similar to those of the peroneal group seen at corresponding periods after primary peroneal suture. Therefore although we have not made the critical comparison with gastrocnemius and plantaris muscles after direct tibial suture it does not seem likely that the factor of crossed innervation greatly affects the course of recovery during the periods examined.

The series of unions of peroneal into tibial includes five cases, and as shown in Table 5 the intervals between the operations were much longer than in the previous series, of which indeed they form a continuation. Biopsy was not performed after

the same interval in all cases, and in one case after 16 months of atrophy the nerves were sutured and the animal left for 10 months recovery. Since the sutures were made at a distance computed as 30 mm. from the centre of m. gastrocnemius, we may calculate that fibres arrived at the muscle after $7+\frac{30+15}{3{\cdot}5}=20$ days. This time has to be added to the times between operation to obtain the total time of atrophy and is also the total period of atrophy of the control primary cross-unions. For the reasons given on p. 29 it is likely that arrival of fibres is somewhat delayed after secondary suture, and therefore one month has been assumed in calculating the times of denervation.

We have, therefore, with primary and delayed sutures, a series in which re-innervation can be studied after periods of muscle atrophy ranging from 1 to 17 months. The state of the various muscles which have recovered after increasing atrophy will now be described.



Text-fig. 9. After 3 months of atrophy and 2 of innervation nerve fibre approaches a plate but seems unable to enter (650*e* 5).

In the animal in which 2 months was allowed to elapse between resection and suture (650*e*) the muscles were subjected to a total period of atrophy of about 3 months and were fixed 2 months after the arrival of the fibres. Though there had been considerable shrinkage of the muscle fibres the atrophy was not profound. In most of the muscle fibres the nuclei still retained their original peripheral position; in a few, central rows of nuclei had been formed. The end-plates remained intact on many, probably most fibres. There were no clear signs that the uninnervated plates were undergoing gradual atrophy. They show a central mass of granular sarcoplasm and peripheral nuclei whose number seems normal. There is perhaps a tendency for the sarcoplasm to become restricted to the centre of the plate, so that the more peripheral nuclei as it were lose contact with it. Many of the plates had been re-innervated (Pl. 8, fig. 83), though others had not been reached, even when fibres passed quite close to them (Pl. 8, fig. 84). In other cases fibres have approached a plate but have not succeeded in making contact with the sarcoplasm (Text-fig. 9).

An attempt was made to compare the proportion of plates which had been reached by new fibres after various periods of atrophy. This estimation is very difficult because it is so hard to recognize the uninnervated plates, and this becomes progressively harder as atrophy proceeds. In the sections of the muscle (650*e*) which had been given 3 months of atrophy and 2 months of re-innervation sixteen plates were studied and eight of them contained no fibres. On the opposite side of the same animal a control primary suture had been made at the time of the second operation. This produced a muscle (650*c*) which had therefore been given 1 month of atrophy and 2 of re-innervation, and in this eight out of ten plates had been innervated. Without studying great numbers of sections it would be difficult to test the significance of these figures, but there can be little doubt that the proportion of old plates which is re-innervated falls rapidly as atrophy proceeds (see p. 38). Moreover, the figures given in Table 2 and Text-fig. 6 show that the number of fibres in the plates is less in the muscles which have undergone the longer atrophy. Many escaped fibres are present, running along between the muscle fibres, and in some places making branches and knobs. The fibres within the end-plates are somewhat thickened, as after primary suture, but not more evidently so than in the plates of the control muscle. Probably the *average* number of fibres per plate would be still lower than indicated in the counts, if proper allowance could be made for the difficulty of finding uninnervated plates.

In fact the situation after 3 months of atrophy is that the muscle fibres are not seriously impaired, many of them retain their end-plates and these plates are often re-innervated, but not so often as after shorter periods of atrophy.

In the muscle examined 2 months after arrival of fibres after a denervation of 5 months (680*a*) the atrophy was much more pronounced, though not yet extreme. A large number of the muscle fibres showed the central rows of nuclei which are a characteristic sign of the onset of the later stages of atrophy. There was considerable development of connective tissue and fat, separating the muscle fibres, whose volume, however, remained considerable. Nerve fibres were abundant in the muscle and many of them had become large and medullated. In spite of the abundant innervation it is now difficult to find old plates which have been reinnervated. From this stage of atrophy onwards it is not possible to find sufficient undoubted re-innervated plates to allow the making of counts. The difficulty of distinguishing plates from other collections of nuclei now becomes acute, though in this and subsequent muscles there are occasionally undoubtedly persisting plates both with and without re-innervation (see p. 36). It is therefore not

possible to say whether the apparent fall in the number of persistent plates is real. Certainly the number of re-innervated old plates has now fallen very low. In fact there has been a considerable change in the process of re-innervation between 3 and 5 months of atrophy. The nerve fibres now run in a very irregular manner throughout the muscle, and in particular they pass for very long distances in a longitudinal direction (Pl. 8, figs. 85, 86). In the figures shown they are mostly running close to the muscle fibres, but in the parts of the muscle where atrophy is more severe they run less regularly in the spaces between the muscle fibres, and they may end here in knobs (Pl. 5, fig. 58).

The nerve fibres wrap around the muscle fibres in a variety of patterns, and give off branches some of which presumably make contact with the sarcoplasm, though it is difficult to be sure of the details of the relationship (Pl. 5, fig. 54). Undoubted end-plates are rare and of very abnormal form. At most they consist of one or two irregular branches (Pl. 5, figs. 53, 57) usually formed as collateral outgrowths from a large fibre. These are probably old end-plates innervated in the usual manner by a portion of the outflow. A few further undoubtedly intact old plates which were not innervated were seen (Pl. 5, fig. 55). The nerve fibres sometimes pass quite near to them without sending in branches. Some fibres make claw-like endings not obviously in relation to any muscle fibre (Pl. 5, fig. 56).

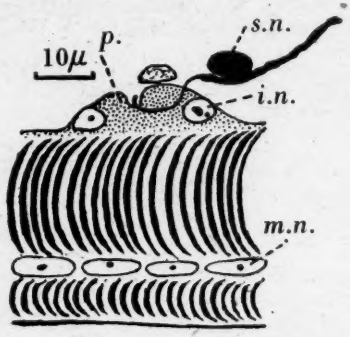
In fact the muscle at this time may be said to differ from one at which the fibres have arrived after only 1 month of atrophy in that (1) there are fewer recognizable end-plates, (2) those which are present contain fewer and generally thicker branches and many of them are probably new plates, and (3) there is a greater abundance of fibres running for long distances between the muscles.

The cause of the differences from the condition found after primary suture is evidently that on account of the further shrinkage of the muscle fibres and the development of collagen between them the streams of axoplasm less often make contact with the old plates. When they do so they find greater difficulty in making entry and hence produce the thickened terminations.

After 7 months atrophy and 2 of re-innervation there is a further development of the above conditions. The muscle fibres had shrunk further and many of them now show central rows of nuclei. But all are recognizably striped muscle fibres, and the whole appearance is of a muscle not yet excessively atrophied, though there is of course a considerable increase of connective tissue and fat (Pl. 8, figs. 87, 88). End-plates are preserved, at least in some of the fibres, and they may stand out laterally as a pronounced hillock. Numerous fibres have returned to the muscle and run for long distances between the fibres. Certainly the majority of these fibres have failed to enter old plates, but a very few were seen which had done so—indenting the sarcoplasm of the old plates (Text-fig. 10). The terminations of the remainder could not be determined. Some of them seem to make complex nets around muscle fibres. Others terminate between muscle fibres, as if blocked in the connective tissue. Occasionally a definite new plate was seen, a fibre ending either as a simple taper, or a lump with a few branches, against the sarcoplasm of the muscle fibre (see p. 33).

The condition is therefore essentially as after 5 months' atrophy, but continued shrinkage of the muscle fibres has further reduced the chances that axoplasmic streams will enter old plates, and re-innervation of these is now very rare.

After 9 months' denervation and 2 of re-innervation some of the muscle fibres are approaching the extreme condition of atrophy in which they are



Text-fig. 10. Nerve fibre making simple contact with the sarcoplasm of the surviving end-plate on an atrophic muscle fibre. 7 months' atrophy, 2 months' innervation (597*a* 3).

reduced to mere threads (Pl. 8, fig. 89). Others, however, still remain of good volume. End-plates are still present, but the nearest approach to re-innervation was that seen in Pl. 5, fig. 60, in which the fibre, in spite of its relatively large size, is clearly having difficulty in entering into contact with the sarcoplasm. In other cases the plate remains quite empty in spite of the presence of nerve fibres near by and even, so far as can be judged, in the sheath which is actually connected with the end-plate (Pl. 5, fig. 59).

Many of the nerve fibres running between the muscle fibres are quite thick and well medullated. Occasionally there are signs that they are entering into contact with the sarcoplasm by throwing off short blunt branches (Pl. 5, fig. 61). Elsewhere the fibres end in little knobs, as if blocked within the connective tissue, even though quite close to the muscle fibre (Pl. 5, fig. 62). Since function had returned in this muscle it must be supposed that

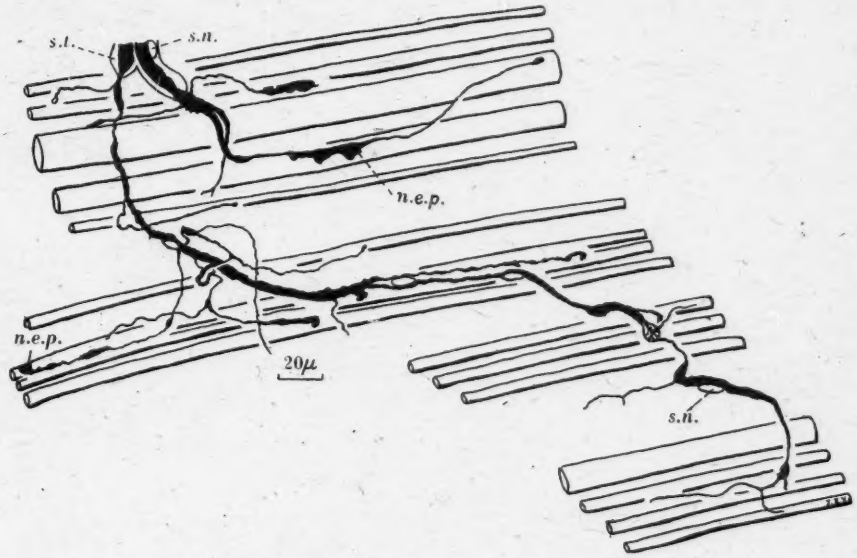
some at least of these formations are able to stimulate the muscle fibres. Very thorough search of many sections revealed only the one re-innervated old end-plate which is shown in Pl. 5, fig. 60, and it must be presumed that after this long period of atrophy functional connexion is made by new contacts. However, at this stage of re-innervation, 2 months after return of fibres, the new plates are still very undeveloped.

Besides the large, presumably motor, fibres there are also in these muscles, as in those after shorter periods of atrophy, elaborate plexuses of fine fibres, probably sensory.

For the study of re-innervation after still longer periods of atrophy we have the muscles innervated by cross-union of peroneal into tibial nerve (see p. 30). In P11 the total time of atrophy was 11 months, but as the animal was sacrified only 2 months after the return of fibres to the muscle the innervation would not be expected to be very complete. However, the nerve bundles in the muscle are well filled with nerve fibres. The atrophy is so severe that in many places the nerve bundles seem to run among a mass of fat cells and blood vessels from which muscle fibres have completely disappeared. Many muscle fibres are indeed evidently approaching the final stage of atrophy, being reduced to narrow threads or strings of nuclei (Pl. 6, fig. 64). At least until the penultimate stages of atrophy the end-plate may persist. Pl. 6, fig. 65, seems to show one in a muscle fibre which is in process of breaking up into separate threads. However, large masses of unstriated sarcoplasm occur rather often on these atrophic muscle fibres (Pl. 6, figs. 64, 65, 68), and it is difficult to be sure which, if any, of them are end-plates. It is impossible to believe that the full structure of the plate could be preserved when the muscle fibre is reduced to a tiny thread with only a fraction of its initial volume.

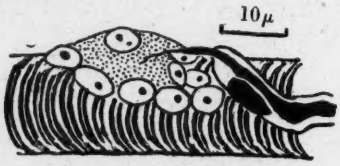
No definitely innervated original end-plates were seen in the many sections of mm. plantaris and gastrocnemius of P11 which were examined. Fine nerve fibres run for long distances between the muscle fibres and sometimes end in knobs. Other single larger fibres in process of medullation, and almost certainly motor, emerge from the ends of Schwann tubes and run across the muscle fibres, branching rapidly and making elaborate plexuses. Short blunt collaterals of these fibres end in simple knobs apparently in contact with muscle fibres, though not obviously related to plates (Pl. 6, fig. 63 and Text-fig. 11). These branches seem to be making contact with the sarcoplasm and building new plates of the most varied shapes, which would explain the appearances seen at longer periods after re-innervation of atrophic muscles (p. 35).

In the two animals in which denervation had lasted for 13 months and innervation for 2 months the atrophy was very profound. Very many of the



Text-fig. 11. Motor nerve fibre branching among muscle fibres and making new end-plates. 11 months of atrophy and 2 of innervation (P11*n* 5).

muscle fibres were narrow threads, often separated from each other by the development of the connective tissue (Pl. 9, figs. 90, 91). Nevertheless, the better-preserved muscle fibres still possess end-plates, and in this case some have been innervated, with indentation of the sarcoplasm in the normal manner (Pl. 6, fig. 66). Such plates even show the excessive darkness characteristic of freshly innervated plates, and their muscle fibres have made a good recovery.



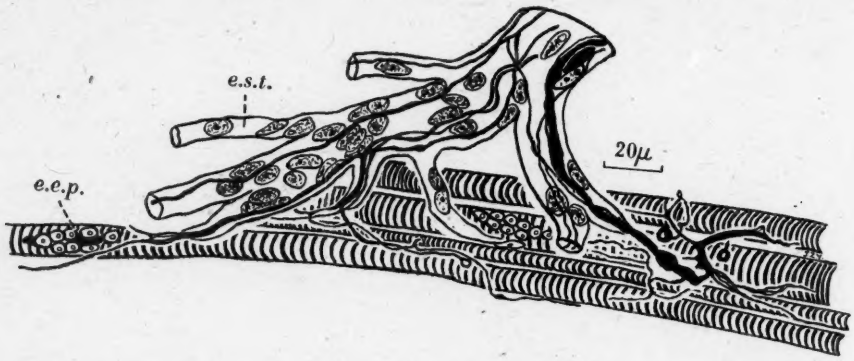
Text-fig. 12. Re-innervation of the surviving end-plate of a very atrophic muscle fibre. 17 months of atrophy and 3 months' innervation (426*p* 1).

in spite of the large volume of the nerve fibre reaching it.

However, large areas of the muscles (gastrocnemius and plantaris) have become so atrophic that little remains except rows of nuclei surrounded by very small amounts of striated substance (Pl. 6, fig. 70). Nerve fibres were less abundant in these regions than in those in which there had been less profound atrophy. The 'perineurial' sheath of the nerve bundles are so thickened that they perhaps prevent the exit of the fibres (Pl. 9, fig. 93).

Nevertheless, nerve fibres of motor type branch out among these masses (Pl. 9, figs. 91, 92) and run for long distances in close contact with even the most atrophic fibres. Although the details of their relations to the sarcoplasm could not be made out with full certainty, there seems every reason to suppose that some of the endings constitute functional contacts.

The muscle fibres which have not reached quite to this extreme stage of atrophy are in process of re-innervation by formation of new end-plates. The



Text-fig. 13. Motor nerve fibre branching and making contact with very atrophic muscle fibres. 17 months' atrophy and 3 months' innervation (426*p* 6).

However, in other large portions of the muscle the nerve fibres run for long distances, as in other atrophic muscles, sometimes branching in a manner suggestive of 'attempts' to make contact with the sarcoplasm, exactly as in the cases already described.

The latest stage of atrophy studied was 17 months, followed by re-innervation for nearly 3 months (426*p*). Even after this time some muscle fibres have a considerable volume. Their end-plates may persist, and in a few cases have been re-innervated (Pl. 6, fig. 69). In this figure the nuclei of the plate can be seen standing out laterally and a nerve fibre is present and has apparently made a simple knob-like ending in contact with the convex surface of the sarcoplasm. Text-fig. 12 shows another old plate in which only a very simple ending has been formed

old plates persist, at least on many fibres, but the old Schwann tubes taper away to fine points at a distance from the end-plates. Text-fig. 13 shows several such tubes some with and some without nerve fibres. The flow of axoplasm evidently becomes undirected where it leaves the end of the tube, so that it fails to enter the end-plate, which now consists only of the closely packed nuclei and a little sarcoplasm. The stream usually divides into several branches at the end of the tube and these wander out across and along and around the muscle fibres in the manner already described, making new end-plates where they come into contact with the sarcoplasm. Some of these new plates already have three or four branches, though still usually only with two to four nuclei. Little collections of darkly staining sarcoplasm appear where the contacts are

made (*p* in Pl. 6, fig. 67), suggesting a reaction between the nerve and muscle fibres. It is difficult to avoid the conclusion that this contact of surfaces produces specific mutual effects.

In one animal (452), after a period of denervation lasting in all for 17 months, a relatively long period of recovery was allowed, the animal being killed 10 months after arrival of fibres at the muscle. In this case, therefore, we can follow the later stages of re-innervation after extreme atrophy. Even in this long time the muscle has by no means fully recovered, and indeed it is clear that it will never be able to do so. There is extensive development of tendon, especially in the periphery of the muscle. Although many of the muscle fibres have made a good recovery of diameter, there are large areas in which few or none are found. Some of these are presumably regions in which there had been development of fibrous tissue and fat during atrophy. Others, however, are clearly regions occupied by much shrunken muscle fibres which have failed to be innervated and to recover their volume. Indeed, the very thin muscle fibres can still be seen in these areas (Pl. 9, fig. 95). Nerve fibres may run among these thin muscle fibres, and we therefore have here a convincing demonstration that delay in re-innervation may make it impossible in large numbers of muscle fibres for restitution of function to occur. In some places, as would be expected, isolated muscle fibres have recovered within an otherwise still atrophic mass.

The actual endings on the muscle fibres are of very strange and abnormal forms, and show the end-product of the process of new formation of plates. The endings often lie in groups as would be expected if they are formed by the development of the branching processes of motor fibres such as those seen in the earlier stages of recovery (text-fig. 11). A single nerve fibre may send lateral branches to supply a number of muscle fibres (Pl. 9, figs. 96, 97). The branches in contact with the sarcoplasm are often of flattened leaf-like form, very unlike those in typical end-plates (Pl. 6, fig. 71). It is difficult to give a count of the number of such endings, since some of them are mere lateral bulges. However, the average number of branches counted was only 2·1 with extremes of 1 and 5. Clearly the main branches are much fewer than in a normal plate, and many plates have only a single branch. Once again it appears that there is little relation between the number or shape of endings and their function. The branches are definitely associated with nuclei and a little sarcoplasm to make a differentiated end-plate (Pl. 6, fig. 71). But the number of nuclei is never large (average 3·5 and extremes of 2 and 6); presumably they are simply the modified muscle fibre nuclei which happened to lie in the neighbourhood of the incoming nerve fibre.

DISCUSSION

The effect of atrophy on re-innervation

Evidently the process of re-innervation is not the same at all stages of muscle atrophy. Functional connexion is made when a suitable motor nerve fibre touches the sarcoplasm of a muscle fibre. Assuming that by the processes occurring at the site of injury some nerve fibres have become directed into channels similar to their original ones it is obviously essential for successful regeneration that they should follow these channels right back to the end-organ.

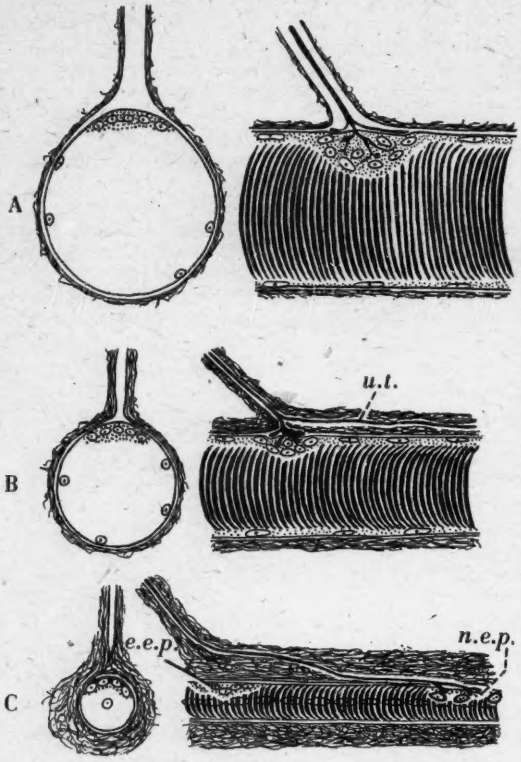
If the new fibres reach the muscle quickly, they enter the old plates and rapidly re-establish a pattern of innervation very similar to that which was originally present. As atrophy proceeds, however, the fibrosis which develops between the muscle fibres interferes with the connexion between the Schwann tube and the old end-plate, so that the returning stream of axoplasm is not wholly directed back into the latter. We do not know exactly what factors control the disposition of the new collagen which produces this effect, but we may imagine its arrangement to be as shown in Text-fig. 14, blocking the end of the Schwann tube (see also Text-fig. 13). At first no doubt the closure is incomplete and channels remain through which the axoplasmic stream can reach the plate. The fact that the branches within the plate may be short and thick seems to indicate that there is a progressive constriction of the space around the surface of the fibre into which the axoplasm can flow. Alternative channels also exist among the strands of collagen, and part of the stream may flow away as an 'ultraterminal fibre'. Ultimately the whole stream deviates in this way and runs along between the muscle fibres to produce the characteristic pattern of the re-innervation of an atrophic muscle.

Sooner or later these 'escaped' fibres may be led into contact with the sarcoplasm of a muscle fibre, where they cause the production of a new end-plate. Their chance of making any contact at all decreases as the muscle fibres shrink and the collagen around them thickens. Moreover, it becomes progressively less likely that a nerve fibre will innervate approximately its original set of muscle fibres. After the longer periods of atrophy the nerve fibres do not even run along the muscle fibres but across them (Pl. 9, fig. 97, etc.). In fact, prolonged atrophy increases the probability of wrong functional connexions by adding to the chances of the nerve union scar further possibilities of confusion in the muscle itself.

These fibres running along and across the muscle fibres are of course new formations not laid down in any existing nervous pathway. Yet they may become of large diameter and fully medullated, and

are provided with Schwann cells. Probably these latter follow over the newly laid fibre in the manner described by Speidel (1932), but Boeke (1916) has suggested that the Schwann cells may precede the nerve fibres.

In any case it is certain that quite new fibres can be laid down; a remarkable instance of the structural



Text-fig. 14. Diagrams to show changes in relation of Schwann tube to muscle fibre as a result of progressive atrophy and fibrosis. On the left the conditions as seen in transverse section before innervation, on the right the result produced when the fibres return (seen in longitudinal section). A. Very little atrophy, space above the end-plate large, returning fibres able to branch in the old plate. B. Space restricted by fibrosis, part of the stream of axoplasm escapes. C. End of tube closed by fibrosis and connexion with old plate broken.

plasticity of the adult mammalian nervous system. The Schwann tubes which mark the course of the old nerve fibre provide a useful guide for the regenerating fibres towards the old end-organ (Holmes & Young, 1942), but the process of atrophy may prevent them from leading the stream all the way back to the latter. Glees (1943) has suggested that the pattern of Schwann tubes is important in the skin for directing fibres back to their end-organs. It would be very interesting to discover whether, with the progress of atrophy, the re-innervation becomes more difficult in the skin as it does in the muscle. It may be that this is one of the chief factors which produces a poor sensory and motor recovery when suture is long delayed. Recovery after any nerve suture is dependent on the chance that suitable central fibres will enter suitable peripheral pathways, and apparently atrophy adds the further hazard that at the end of its journey the fibre may not reach the original end-organ.

Do the end-plates disappear?

End-plates have been identified on muscle fibres in all but the most extreme stage of atrophy, and there was evidence of some re-innervation of old plates even when the nerve fibres arrived after 17 months of denervation. There is therefore no proper evidence to show that end-plates atrophy. Moreover, there is no evidence of any change in the number of nuclei in the plate, even of the much atrophied fibres in Text-fig. 13. However, the outer nuclei which are probably those of fibroblasts increase in the early stages. Unfortunately it is rather difficult to find the uninnervated plates of the later stages of atrophy, so that no proper counts can be made to discover whether there is a gradual reduction in the number of nuclei or other sign of atrophy. For the same reason it is still more difficult to estimate the proportion of plates to muscle fibres and hence to discover whether any are disappearing. The fact that some plates persist for a long time does not prove that others have not disappeared. However, in the absence of any evidence that they do so we may assume that they all remain during the early stages of atrophy. The final stages of muscle atrophy are still not fully understood (see Tower, 1935, 1939). The fibres become reduced to very narrow strands containing a simple series of nuclei, and in this state the plates can hardly be preserved. Probably many muscle fibres finally break up into separate strands (Pl. 6, figs. 64, 65) and then disappear altogether.

We must conclude, then, that the end-plate remains with relatively little change on most of the fibres for a considerable period (at least 9 months) of atrophy. But its value is reduced much earlier than this by the fact that the fibrosis around the fibre has made it difficult for a new nerve fibre to reach it.

Formation of new end-plates

The streams of axoplasm which run along between the muscle fibres may end blindly in the collagen. If they come into contact with the surface of the sarcoplasm they can cause differentiation of

the latter to produce an end-plate. Such contact may not be made until the fibres have run for long distances. In other cases the fibres emerging from the ends of the Schwann tubes in the muscle wrap around the muscle fibres in the immediate neighbourhood. This may produce a pattern of re-innervation by the formation of new plates which is not greatly different from that produced in normal development. It is particularly in the re-innervation of muscles in the latest stages of atrophy that such an arrangement is found; in the earlier stages the nerve fibres tend to run for longer distances between the muscle fibres. In this respect the muscle may be said to 'return to an embryonic condition' in its last stages of atrophy, and re-innervation of very atrophic muscle may produce a general pattern more similar to the normal than that produced after moderate atrophy (compare Pl. 9, figs. 94 and 96 with Pl. 8, fig. 88).

It is not possible to be certain what determines the making of a contact between nerve fibre and sarcoplasm. Probably it is mainly the mechanical conditions imposed on the streams of axoplasm by the connective tissue between the muscle fibres. Presumably contact is only effective if the muscle fibre is not already innervated though we know little of how innervation confers this immunity and in some cases there is evidence indicating that more than one regenerating nerve fibre makes contact with a muscle fibre (Pl. 2, fig. 21 and Pl. 5, fig. 53). At its simplest a new plate is distinguished simply as a small collection of unstriped sarcoplasm staining brown with silver (Pl. 6, fig. 67). Boeke (1916) shows several examples of such simple 'plates' and, as he says, they may be collateral or terminal. Further development of the plate is produced by collection of some of the nuclei in the neighbourhood, and perhaps also of some fibroblast nuclei to make 'outer end-plate nuclei' (Pl. 3, figs. 28, 29). In this way quite a complete little plate may be built, with the branches of the nerve fibres indenting the sarcoplasm as in a normal plate. In the animal (452) in which we allowed a period of atrophy of 17 months and then re-innervation for 10 months many of the new plates contained five or six nuclei. This suggests that some further process of addition of nuclei and multiplication of nerve branches takes place in the later stages. But Pl. 6, fig. 71, shows that even a long time after suture these new plates remain very unlike normal plates, probably they never reproduce exactly the original condition. It would be very interesting to examine the physiological properties of these products of adult development. There is no doubt whatever that the contact of axoplasm with sarcoplasm produces a differentiation of the latter. This may not seem surprising since we know that it occurs during development, but it is worth emphasis as one of the few undoubted cases in which nerve fibres produce definite structural differentiation in the mammal.

There is presumably a reciprocal effect of the contact on the nerve fibre, which stops its further advance and spreads out into branches over the surface of the sarcoplasm. It may be that this is mainly a simple mechanical effect of the conditions on the flow of axoplasm. But it is not unlikely that more specific factors are also involved, perhaps as a result of the molecular interactions of the surface membranes of the nerve and muscle fibres.

Is there attraction of the nerve fibres to the sarcoplasm?

In the above account of the process of regeneration it has been assumed that the stream of axoplasm flows along over such surfaces and in such tubes as it finds available. Many authors have supposed that the direction of flow is influenced by specific 'neurotropic' attractions (for discussion see Young, 1942). Thus Tello (1907) interpreted the appearances of 'escaped' fibres (p. 22) by supposing that as the fibre was growing past the end-plate a branch was 'attracted' in to the latter. No reliable evidence of such attractive substances has ever been produced (see Weiss, 1934), and there is therefore no reason to postulate their existence unless to explain any otherwise incomprehensible appearances. We have not seen any connexions which could not have been made by the leading back of the stream of axoplasm in the manner already described.

The most difficult part of the process to understand is the final contact with the sarcoplasm. The Schwann tubes and the spaces among the collagen around the muscle fibres provide adequate conductors to lead the new fibres back near to the sarcoplasm, but what determines that the stream should spread out and branch, and then, usually, proceed no further? Since our general assumption is that axoplasm flows forward in streams wherever it is able to do so, we require a special explanation of why it should stop when in contact with sarcoplasm. Mechanical obstruction may be the only factor operating, but it is not fantastic to suppose that there are specific molecular attractions between the muscle surface and axon surface, which keep them in contact. In this connexion it is especially interesting that sensory nerve fibres fail to make connexion with end-plates, even when they reach them (Gutmann, unpublished).

Normalization of end-plates and resorption of excess fibres

The process of regeneration continues for a long time after the arrival of fibres in the plates. Table 2 and Text-fig. 6 show that new branches are formed within the plates until approximately the normal

number is re-formed. In later stages there may even be an excess over normal and perhaps the addition of some nuclei (p. 26). However, it may be that there is a gradual increase in these features of the plates during the lifetime of every individual. The regenerated plates can often be distinguished from normal by the greater thickness of their branches, even one year after suture. There is, however, no evidence as yet that these differences in number and shape of the endings produce functional abnormalities. We have seen that the first fibres to reach the end-plates are very thin, but are able to stimulate the muscles, though not necessarily with full effectiveness. It would be very interesting to discover whether there are any special features of the transmission associated with either these first connexions with old plates or the new plates which are formed in the more atrophic muscles.

The course of the increase in number of branches (Text-fig. 6) seems to be similar after the various types of lesion, but to become slower and slower with increasing periods of denervation. After more than 5 months of atrophy the re-innervation is so abnormal that counts of this sort become impossible, the branches formed in the new end-plates remaining few in number and irregular in shape. If *any* contact between nerve fibre and sarcoplasm is adequate for transmission these abnormalities may be functionally unimportant, but it is not impossible that the slow normalization in these respects is a factor tending to produce poor recovery after long denervation.

The converse process of normalization by reduction of irregularly connected fibres has often been suggested but never very convincingly demonstrated (Boeke, 1916). Such excess fibres are of two main types: (1) sensory and sympathetic fibres which have proceeded along motor pathways into the muscle, (2) motor fibres which have failed to enter their end-plates and proceeded as 'escaped fibres' among the muscles. Fine networks interpreted as sensory have been seen after all operations, more frequently after severance and suture than after crushing the nerve (p. 29). Some are seen at the latest stages (one year) after suture. We are unable to say for certain whether there is a gradual absorption, though we have the impression that such nets are less prominent in the later stages.

Escaped motor fibres can certainly grow to a large size and become medullated. We have seen them at the longest periods after suture, and again, therefore, cannot assert that they are resorbed. But the impression given by the later stages is that more of the plates are terminal, as if some of the ultra-terminal fibres had been removed. But some very irregular forms may persist into the latest times examined (Pl. 3, figs. 34–37). The whole question of the trophic dependence of nerve fibres on their function still remains completely obscure.

Boeke (1916) has emphasized that in the early stages of regeneration there is an 'excessive' re-innervation of the muscle. In so far as it refers to the actual connexions with the muscle fibres this is the reverse of true. The earliest end-plates contain very few branches and thereafter the number gradually increases (Text-fig. 6). But since many motor fibres 'escape', and many sensory fibres are present, there may be an unusually large number of nerve fibres in the muscle. As stated above we are inclined to agree with Boeke that this excess is later reduced, but some quantitative treatment is necessary before any definite statement can be made.

Delay between return of fibres and onset of muscle function

In the series in which the nerve was crushed close to the muscle we have shown that fibres arrive back near the end-plates about 12 days after the operation, whereas electrical stimulation of the nerve produced contraction first on the 18th day and reflex functioning appeared on the 23rd day. It seems that there is no great delay between the actual entry of fibres into the end-plates and the onset of their power to function. Table 4 shows that the delay between arrival of fibres and the onset of functioning is greater after suture than after crushing the nerve and, so far as we can determine, steadily increases with increasing periods of atrophy. We may suppose that this increasing delay with longer atrophy is due to the longer time taken to make the necessary number of connexions with old end-plates and to form new ones.

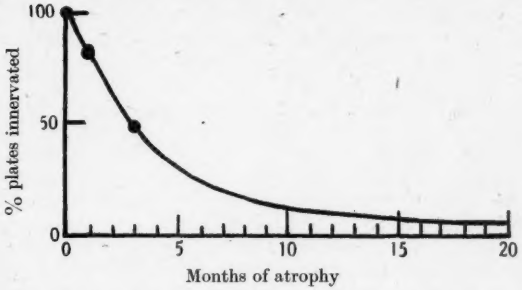
Effect of atrophy on the success of re-innervation

For many reasons the recovery of a muscle becomes increasingly more difficult as its atrophy proceeds. The most rapid innervation is produced when new fibres proceed directly into the old plates. This may occur even in the later stages of atrophy, but becomes progressively less frequent. It would be very interesting to estimate the extent to which old and new plates participate in the innervation after various periods of atrophy. Our data are not complete enough to allow a very exact estimate. Re-innervation after crushing close to the muscle probably proceeds wholly by connexion with the old plates. After crushing further away some new plates are found (p. 25). After immediate suture, where the fibres return to the muscle within about 1 month, most of the re-innervation is by connexion with old plates, but some of the latter fail to be innervated. For instance, in muscle 650*c*, examined 2 months after return of the fibres, two out of ten plates examined contained no fibres (p. 31). But in muscle 650*e*, on the opposite side of the same animal, there had been denervation for 3 months

and re-innervation for 2 months, and only five out of ten plates contained fibres.

With the later stages of atrophy the shrinkage of the muscle fibres makes it so difficult to find the plates that no similar counts are possible. A few re-innervated old plates were found in nearly all muscles, even after 17 months of atrophy. But from stages with 7 months of atrophy onwards they were very rare. We may conclude that the curve for the proportion of old end-plates which are re-innervated runs somewhat as in Text-fig. 15. But it must be emphasized that the shape of the curve is only guessed from considerations of the above facts —an exact estimation of it remains to be made.

Since it is possible for new plates to be formed it might be argued that it is of little importance for the recovery of the muscle whether old or new plates are used. But we have already seen that the



Text-fig. 15. Curve to show the percentage of surviving end-plates which become re-innervated during recovery after various periods of atrophy. Insufficient data are available to allow a full plot to be made, and the curve is therefore only a rough approximation illustrating the general impression obtained from study of the process of re-innervation.

process of recovery becomes slower and less successful as the atrophy becomes more severe. A muscle which has been allowed to atrophy for 9 months shows fewer end-plates and smaller muscle fibres 3 months after innervation than does one which has atrophied only for 3 months. We do not know whether this slower recovery of the muscle fibres which become innervated prejudices the ultimate recovery of diameter and power of contraction. Certainly the pattern of innervation becomes progressively less normal after increasing periods of atrophy and therefore the likelihood of normal functional reconnexions decreases (Cooper, 1929). Even more serious is the fact that when re-innervation proceeds by the formation of new end-plates many muscle fibres fail to become innervated at all. This is best seen in animal 452 where on one side fibres returned after 1 month of atrophy, and on the other side after 17 months. The muscles were examined 10 months after return of the fibres, so that there was full opportunity for the muscles to recover. On the side where there had been atrophy of only 1 month they had done so. On the more atrophied side, however, not only were the innervated muscle fibres *still* abnormally small, but *there were areas in which the muscle fibres had failed to recover, in spite of the presence of nerve fibres among them* (Pl. 9, fig. 95). In fact this muscle, even after 10 months, made a very much less complete recovery than its less atrophied fellow. The difference is well shown by Text-fig. 16 and by the weight of the gastro-

Text-fig. 16. Photographs of the gastrocnemius muscles of rabbit 452 which had been innervated for 10 months after denervation of 1 month in case of A, 17 months in B.

cnemius, which was 2·92 g. on the side with only 1 month of atrophy, 2·02 g. on that with 17 months of atrophy. We do not know whether the process of recovery was still proceeding as late as 10 months after suture, but there is every reason to suspect that the defect produced by atrophy would never be made up, since many muscle fibres had disappeared altogether and others were unable to respond even though nerve fibres branched among them (Pl. 9, fig. 95).

We see then that, apart from the actual disappearance of muscle fibres and all the other difficulties imposed by long atrophy, it produces irreversible changes in the muscle and severely

prejudices the final recovery. That this can occur also in man is shown by a case in which the radial nerve was sutured after it had been interrupted 5 years previously. One year after suture no recovery had taken place and examination of a portion of the m. extensor carpi radialis brevis showed that although abundant nerve fibres were present they had failed to make connexions with the much-atrophied muscle fibres.

This is an extreme case, and we do not know how far similar factors will operate to reduce the effectiveness of recovery after more moderate periods of atrophy. However, since the lengths of nerve to be regenerated are often great, it is not uncommon in man for a muscle to remain denervated for a year, even when suture is performed within a few months of injury. If the muscles atrophy at approximately the same rate as those here studied it seems likely that their recovery after such long periods of atrophy can be at best only partial. At least three factors will combine to ensure this. (1) The establishment of new connexions becomes more difficult so that the rate of recovery is reduced, and there is a great increase in the delay between return of fibres and recovery of function. (2) The pattern of innervation becomes more and more abnormal, so that the chances of correct connexions are reduced, especially in mixed nerves. (3) The number of muscle fibres which never become re-innervated is increased. It is not yet possible to say exactly what period of denervation will make these effects serious in man. They all operate increasingly from the beginning of denervation and perhaps begin to be severe after 6–9 months. But everything which can be done to reduce the time between injury and return of fibres to the muscles assists the process of regeneration and is likely to improve the final functional result.

SUMMARY

1. In normal motor end-plates the nerve fibres make contact with the surface of the sarcoplasm. They may indent the latter but are not surrounded by it, and a membrane always separates the insides of the nerve and muscle.

2. In the peroneal muscles of the rabbit the plates contain an average of 5·8 terminations, mostly tapering to fine points. Within the sarcoplasm of the plate is an average of 8·1 nuclei belonging to the muscle fibre. Outside the plate lie one or two fibrocytes. A few Schwann nuclei may also lie near the plate.

3. After interruption of the nerve there is no increase in the number of nuclei within the sarcoplasm of the plate, but the outer fibrocytes increase in number. When a muscle fibre shrinks its end-plate may remain intact, though shrunken, even after more than a year of denervation.

4. After a nerve has been interrupted by crushing close to a muscle one nerve fibre returns to each plate and rapidly branches in contact with its sarcoplasm.

5. After the longer periods of atrophy imposed by crushing the nerve at a distance from a muscle a portion of the returning stream of axoplasm often 'escapes' and makes an ultra-terminal fibre. Some end-plates are not re-innervated and new plates are formed where the ultraterminal fibres touch the sarcoplasm.

6. After severance and immediate suture of the nerve these tendencies are exaggerated, and in addition many small fibres (presumably sensory and sympathetic) enter the muscle. Here they do not form end-plates but 'escape' to form elaborate networks along the muscle fibres. When a large and a small fibre approach a plate only the former enters it.

7. The number of endings in the plates returns to normal at about 70 days after a nearby crushing of the nerve, 100 days after a more distant crush. It is still subnormal even 1 year after severance and suture.

8. The delay between return of fibres to the muscle and the onset of reflex functioning is 11 days after the nerve has been crushed close to the muscle, 22 days after a more distant crush injury, and 25 days after severance and suture close to the muscle. After delayed suture involving increasing periods of denervation this delay increases and reaches 55 days after 9 months of atrophy.

9. The endings in many of the plates remain somewhat abnormal at long periods after any form of nerve injury. The excess of ultraterminal fibres and the networks of fine fibres may remain a year after suture. Perhaps they are gradually reduced, but only very slowly.

10. When muscles have been kept denervated for increasing periods the proportion of old end-plates which becomes re-innervated is progressively reduced. Most of the nerve fibres escape and run along between the muscle fibres, ultimately making contact with the sarcoplasm and forming new plates. In the later stages of atrophy they tend to run across the muscle fibres.

11. With increasing periods of atrophy the effectiveness of re-innervation is prejudiced by at least three factors: (*a*) the increasing difficulty and slowness with which new plates are formed; (*b*) the abnormality of the pattern of re-innervation, increasing the likelihood of wrong connexions; (*c*) the fact that many muscle fibres are never re-innervated at all.

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EXPLANATION OF PLATES

Except where stated all figures are of peroneal muscles of the rabbit stained with Bielschowsky's method. Animal reference numbers are given for each figure.

Key to Lettering

a.m. atrophic unrecovered region of muscle; *b.v.n.* blood vessel nerves; *cap.* capillary; *e.* blind termination; *e.p.* end-plate; *e.e.p.* empty end-plate; *f.* fibrocyte; *fa.* fat; *i.n.* inner end-plate nucleus; *k.* terminal knob; *n.f.* new nerve fibre branching in muscle; *o.n.* outer end-plate nucleus; *p.* protoplasm of end-plate; *s.* small (? sensory) nerve fibre; *sh.* thickened sheath of nerve bundle; *s.n.* Schwann nucleus; *u.s.* unstriped sarcoplasm of atrophic muscle fibre; *u.t.* ultraterminal fibre.

Plate 1. Normal end-plates

Fig. 1. Surface view showing how the branches indent the sarcoplasm. (732*c* 3.)

Figs. 2, 4. Lateral views showing how the branches lie between the inner and outer end-plate nuclei. The branches indent the sarcoplasm but are not wholly surrounded by it. (732*c* 6 and 702*f* 1.)

Fig. 3. Surface view. (733*e* 5.)

Fig. 5. Lateral view showing tapering and bulbous terminations. (702*f* 1.)

Fig. 6. Lateral view showing branching some distance from the muscle fibre and several Schwann nuclei near the plate. (702*f* 1.)

Fig. 7. Lateral view of the same condition. Note also the clear channels in the sarcoplasm around the terminal branches. (727*h* 1.)

Fig. 8. End-plate in which two branches have joined, forming a complete ring. (733*e* 5.)

Plate 2. Re-innervation after crushing the nerve 20 mm. from its entry to the muscle. The time before and after the first arrival of fibres at the end-plates is given after each figure number *in days.*

Figs. 9–11. End-plates which have not yet been innervated, 9 and 10 in lateral, 11 in surface view. (711*b* 1, 711*f* 3 and 734*b* 1.)

Fig. 13. Finest fibre just entering plate. (734*b* 1.)

Fig. 14. New branches within the plate. (710*h* 3.)

Fig. 15. A single fibre innervating two plates in series and forming an ultraterminal fibre. (710*h* 2.)

Fig. 16. Terminal loops in regenerating plate. (710*h* 2.)

Fig. 17. Plate with ultraterminal fibre. (733*a* 5.)

Fig. 18. The ultraterminal fibre persists even 3 months after re-innervation. (695*a* 1.)

Fig. 19. Even in the earlier stages each end-plate is innervated only by one fibre. (733*a* 4.)

Fig. 20. Network of fine (? sensory) fibres persisting 3 months after re-innervation. (695*a* 4.)

Fig. 21. End-plate innervated by two fibres approaching from opposite directions. (695*a* 6.)

Fig. 22. End-plate 6 months after re-innervation, showing abnormally thick terminations. (706*c* 4.)

Plate 3. Re-innervation after crushing the nerve 100 mm. from the muscle. The time before and after arrival of fibres at the end-plates is given after each figure number *in months.*

Fig. 23. A portion of the axon has entered the end-plate, but the greater part forms an ultraterminal fibre. (727*a* 4.)

Fig. 24. As fig. 23, but with ring-like endings. (726*a* 6.)

Fig. 25. As fig. 23. The ultraterminal fibre has continued to thicken but the end-plate remains very under developed. (696*a* 2.)

Fig. 26. Network of (? sensory) fibres. (727*a* 4.)

Fig. 27. End-plate without ultraterminal fibre. One process much thickened. Note that the branch seen in lateral view is separated from the surface of the end-plate sarcoplasm by a very narrow space. (696*a* 1.)

Fig. 28. New end-plate of very simple form. (696*a* 4.)

Fig. 29. A more developed new end-plate, formed on the end of the ultraterminal fibre of another plate (not shown).

Fig. 30. Terminal rings on the end of 'escaped' fibres. They are close to a muscle fibre but have not caused the development of end-plates. One of them has become detached from its nerve fibre and is presumably degenerating. (727*a* 1.)

Fig. 31. Ending of escaped fibre in endomysium. Note also the atrophic muscle fibre with a central row of nuclei. (727*a* 1.)

Fig. 32. Undeveloped end-plate showing swelling of axon proximally presumably due to resistance to its entry to the plate. A collateral branch ends in the endomysium. (696*a* 1.)

Fig. 33. Plate remaining undeveloped even after re-innervation for 3 months. (702*e* 2.)

Figs. 34, 35. Over-developed plates found after re-innervation for 5 months. Note large number of thick branches. In fig. 35 a branch has apparently left the plate and run back along the entering fibre. (703*k* 1.)

Fig. 36. Another atypical plate after 5 months, with a large and small loop. The large loop definitely has no protoplasmic centre and must have been formed by the union of two streams of axoplasm. (703*k* 4.)

Fig. 37. Atypical plates still present 8 months after re-innervation, showing thick terminal branches and a collateral mass which, though not in contact with a muscle fibre has not been resorbed. (704*g* 4.)

PLATE 4. Re-innervation after immediate suture 48 mm. from the muscle. The time before and after return of fibres to the region of the end-plates is given after each figure number *in months*.

Fig. 38. Broad irregular branches within the end-plate and complex ultraterminal fibres. (753*d* 9.)

Fig. 39. Besides the large fibre running into the end-plate a small one also runs in the same tube, turns back and ends blindly. Note also the rings within the plate. From a union of central tibial with peripheral peroneal stump. (581*h* 1.)

Fig. 40. Simple plate 2 months after return of fibres. (708*e* 9.)

Fig. 41. Small branches running into end-plate and large ultraterminal fibre ending blindly. (708*e* 6.)

Fig. 42. Plexus of presumably sensory fibres. (650*c* 5.)

Fig. 43. Intramuscular nerve 35 days after crushing the main trunk 100 mm. from the muscle. For comparison with fig. 44. (779*a* 4.)

Fig. 44. Intramuscular nerve from the opposite side of the same animal as shown in fig. 43, a suture having been made 48 mm. away, 35 days previously. Fibres present only in some of the tubes. (779*g* 4.)

Fig. 45. Loop in end-plate. The arms of the cross are in complete continuity at the junction. (650*c* 1.)

Fig. 46. Collateral knob on an ultraterminal fibre. Such collaterals form new plates when they meet the sarcoplasm of a muscle fibre. (650*c* 5.)

Fig. 47. Large ultraterminal fibre which has not been absorbed even 4 months after arrival of fibres at the muscle. (762*f* 6.)

Fig. 48. Regenerated end-plate of approximately normal appearance. (671*j* 4.)

Figs. 49, 50. End-plates still very simple 6 months after return of fibres to the muscle. (752*g* 1.)

Fig. 51. End-plate with thickened branches 6 months after return of fibres. (752*g* 1.)

Fig. 52. Networks of fine fibres persisting 9 months after return of fibres. (452*a* 1.)

PLATE 5. Re-innervation after delayed suture of tibial into peroneal nerve. The time before and after return of fibres to the end-plates is given after each figure number *in months*.

Fig. 53. Simple new end-plate; nearby the ending of another fibre on the same muscle fibre. (680*a* 1.)

Fig. 54. Simple collateral ending perhaps formed by the innervation of an old plate. (680*a* 1.)

Fig. 55. Surviving end-plate with nerve fibre passing by but not making a connexion. (680*a* 5.)

Fig. 56. Irregular claw-like terminations. (680*a* 3.)

Fig. 57. Two well-formed but simple terminal endings. (680*a* 7.)

Fig. 58. Ending in endomysium, not connected with a muscle fibre. (680*a* 1.)

Fig. 59. Surviving end-plate remaining empty in spite of presence of numerous nerve fibres in the neighbourhood. (581*a* 5.)

Fig. 60. Surviving end-plate into which new fibre has just penetrated without making any branches. (581*a* 6.)

Fig. 61. Simple terminations of fibres running for long distances along muscle fibres. (581*a* 5.)

Fig. 62. Simple endings probably not in contact with muscle fibres. (581*a* 6.)

PLATE 6. Re-innervation after delayed suture of peroneal into tibial. Figures are of gastrocnemius or plantaris muscles, and times before and after return of nerve fibres to end-plates are given after each figure number *in months*.

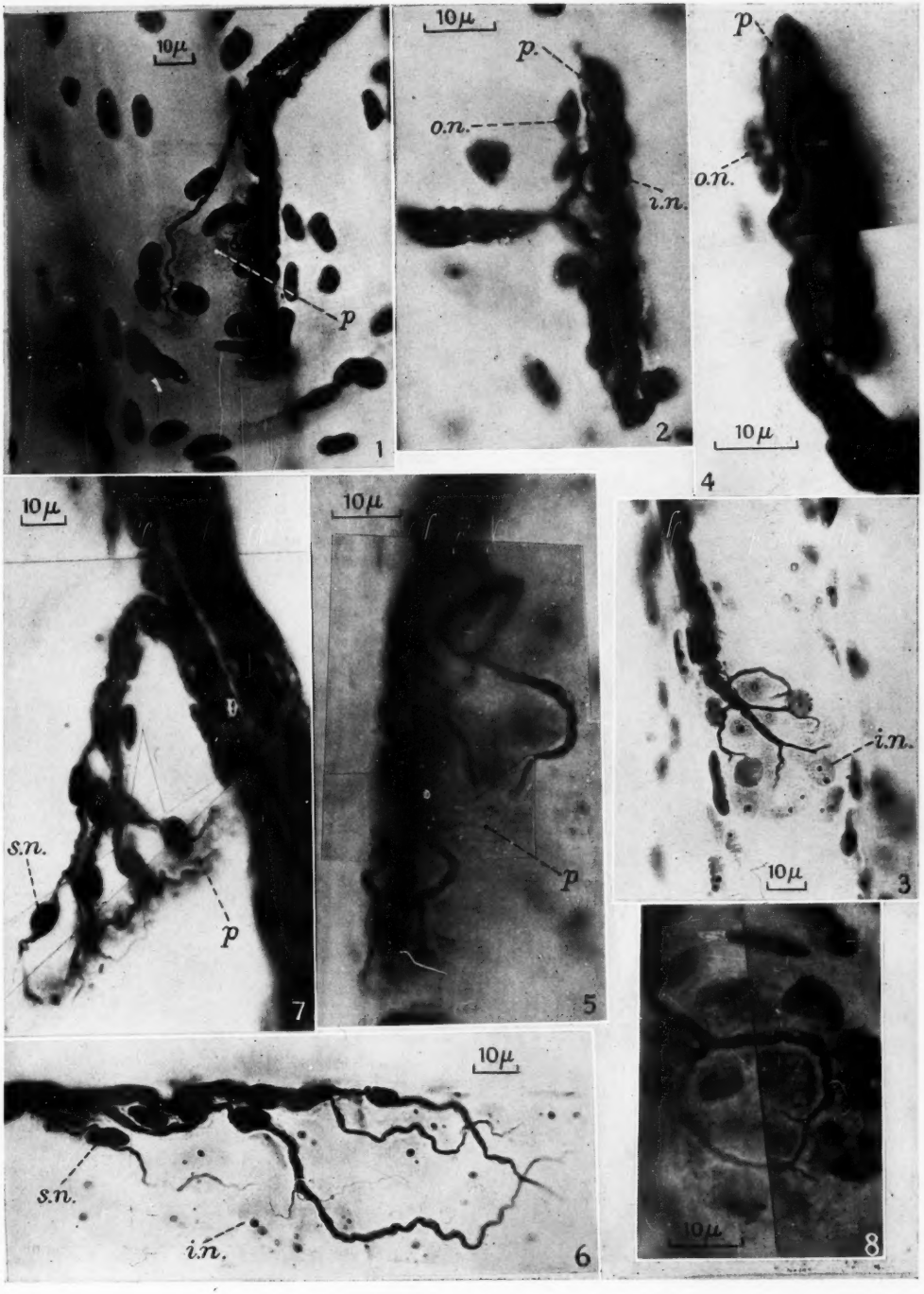
Fig. 63. Nerve fibre running along muscle fibre and showing various dilations in contact with the sarcoplasm. (P11*n* 5.)

Fig. 64. Muscle fibres remaining very atrophic even some time after return of fibres. (P11*l* 2.)

Fig. 65. Muscle fibres remaining very atrophic and apparently in process of division into separate strands. A large mass of unstriated sarcoplasm which might be the remains of an old plate is seen (see, however, fig. 68). (P11*l* 7.)

Fig. 66. Nerve fibre innervating a probably surviving plate and then passing on as an ultraterminal fibre to make new endings. (P10*g* 4.)

Normal end plates

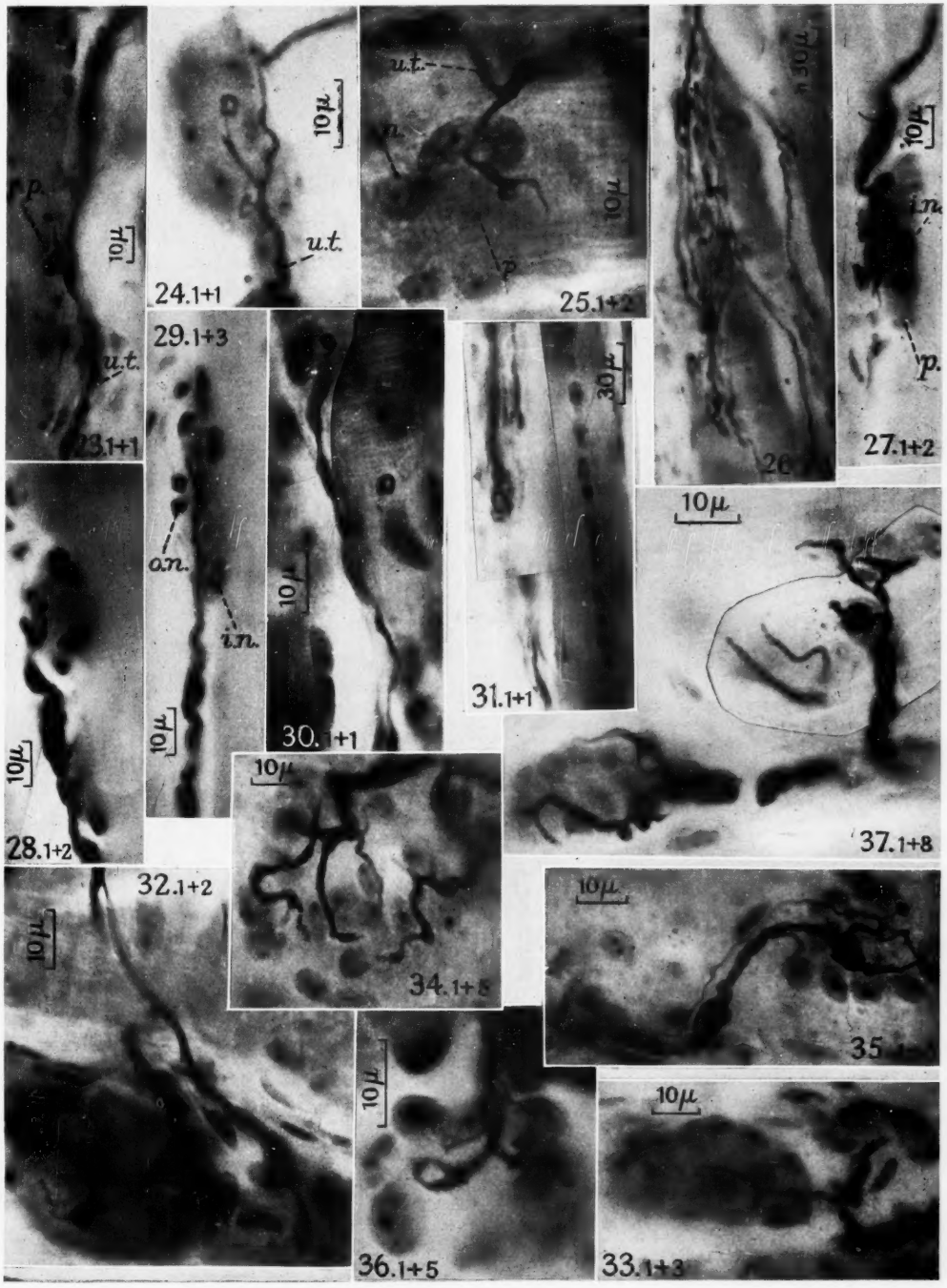


Figs. 1–8

Nerve crushed 20 mm. from muscle. Time in days.

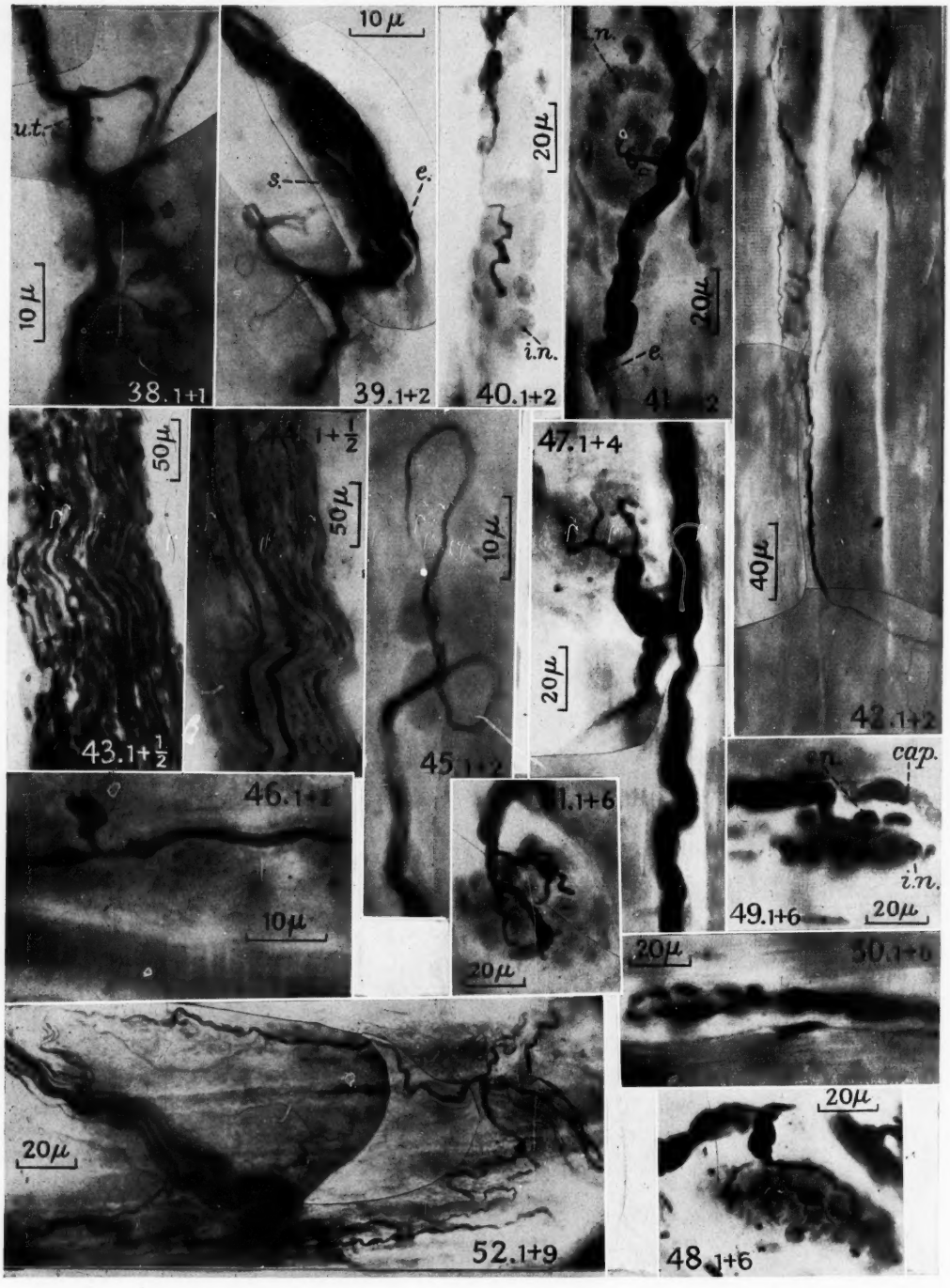
Figs. 9–22

Nerve crushed 100 mm. from muscle. Times in months.



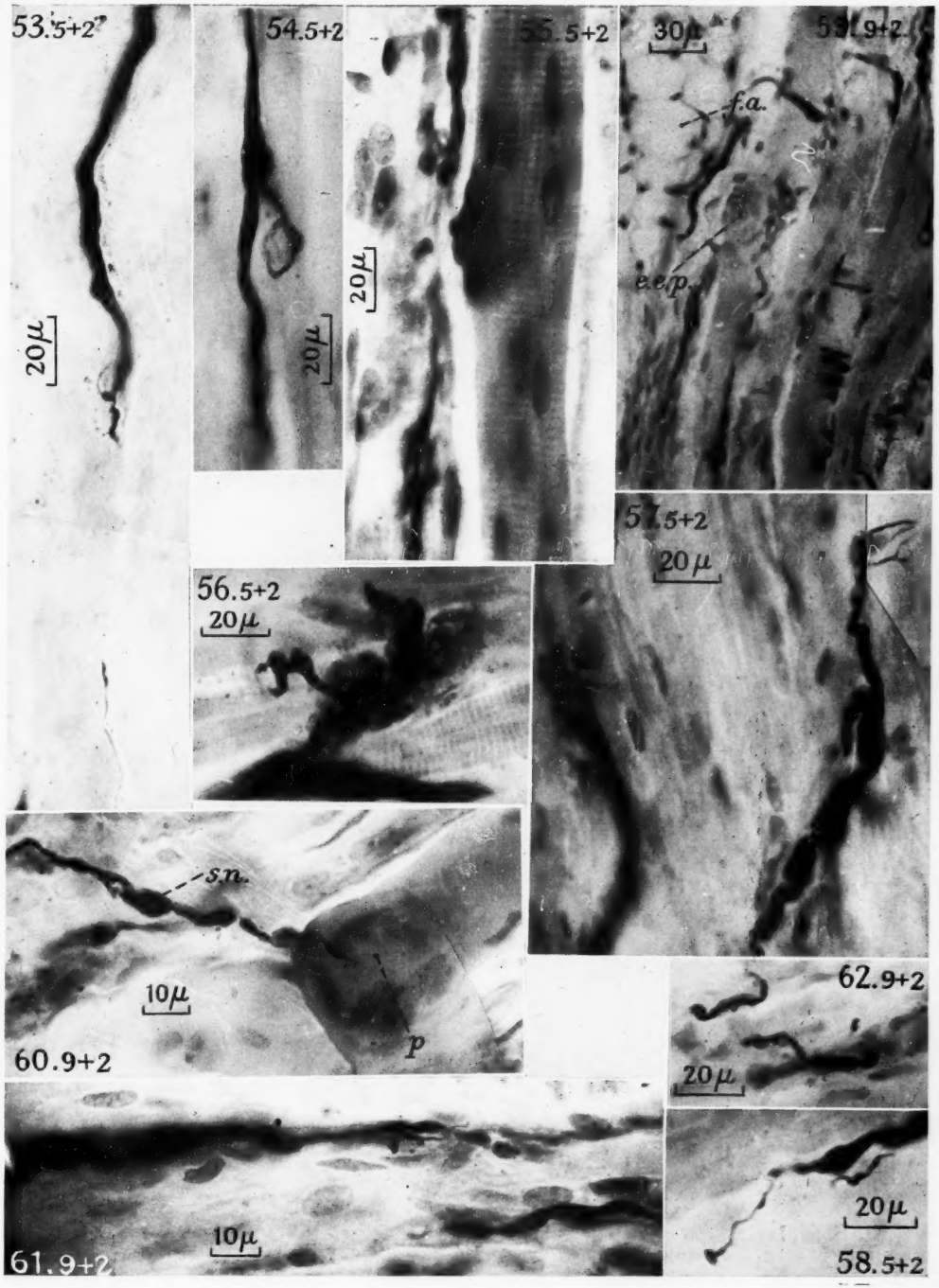
Figs. 23–37

Immediate suture. Times in months.



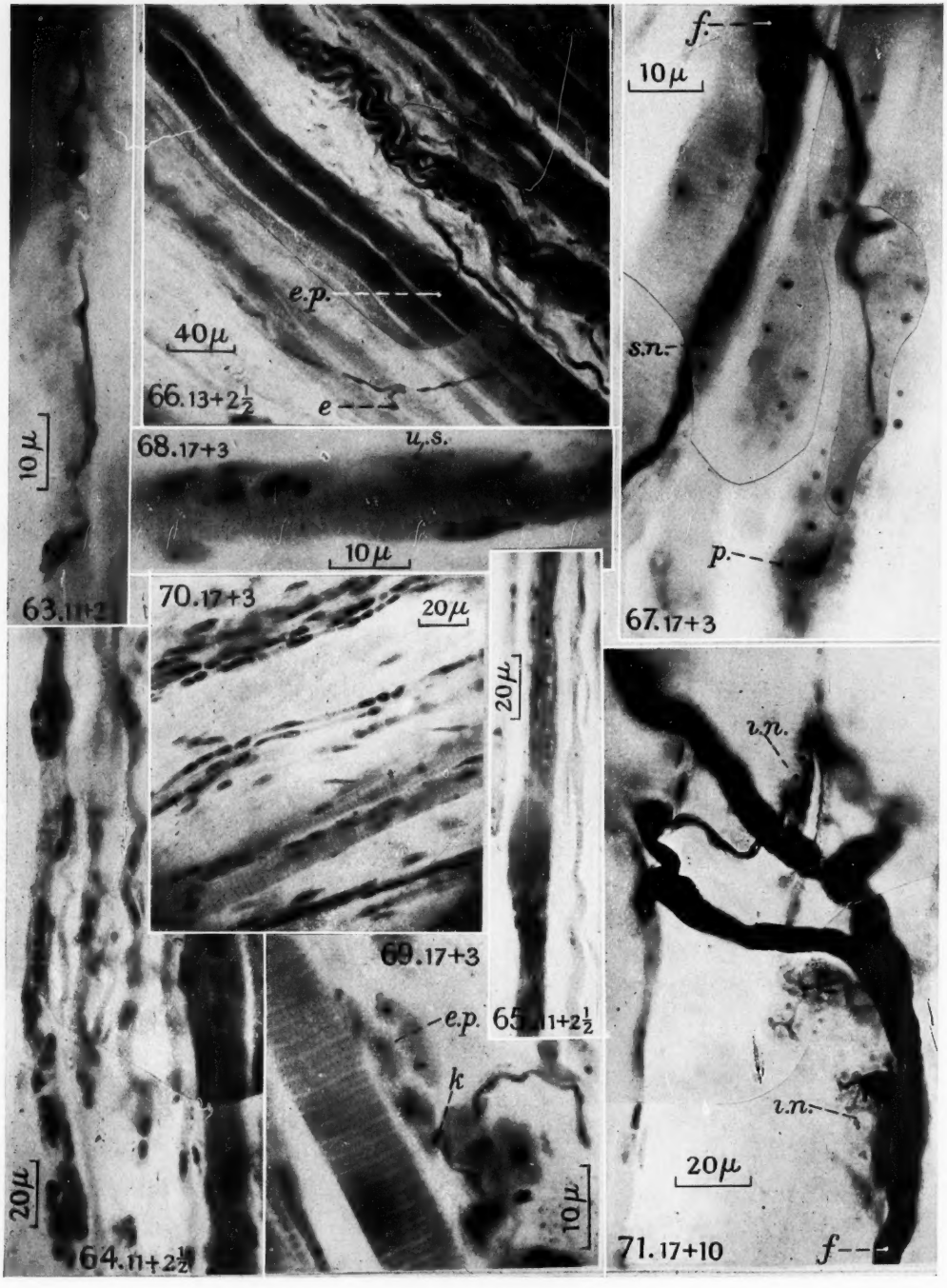
Figs. 38–52

Delayed suture. Times in months.



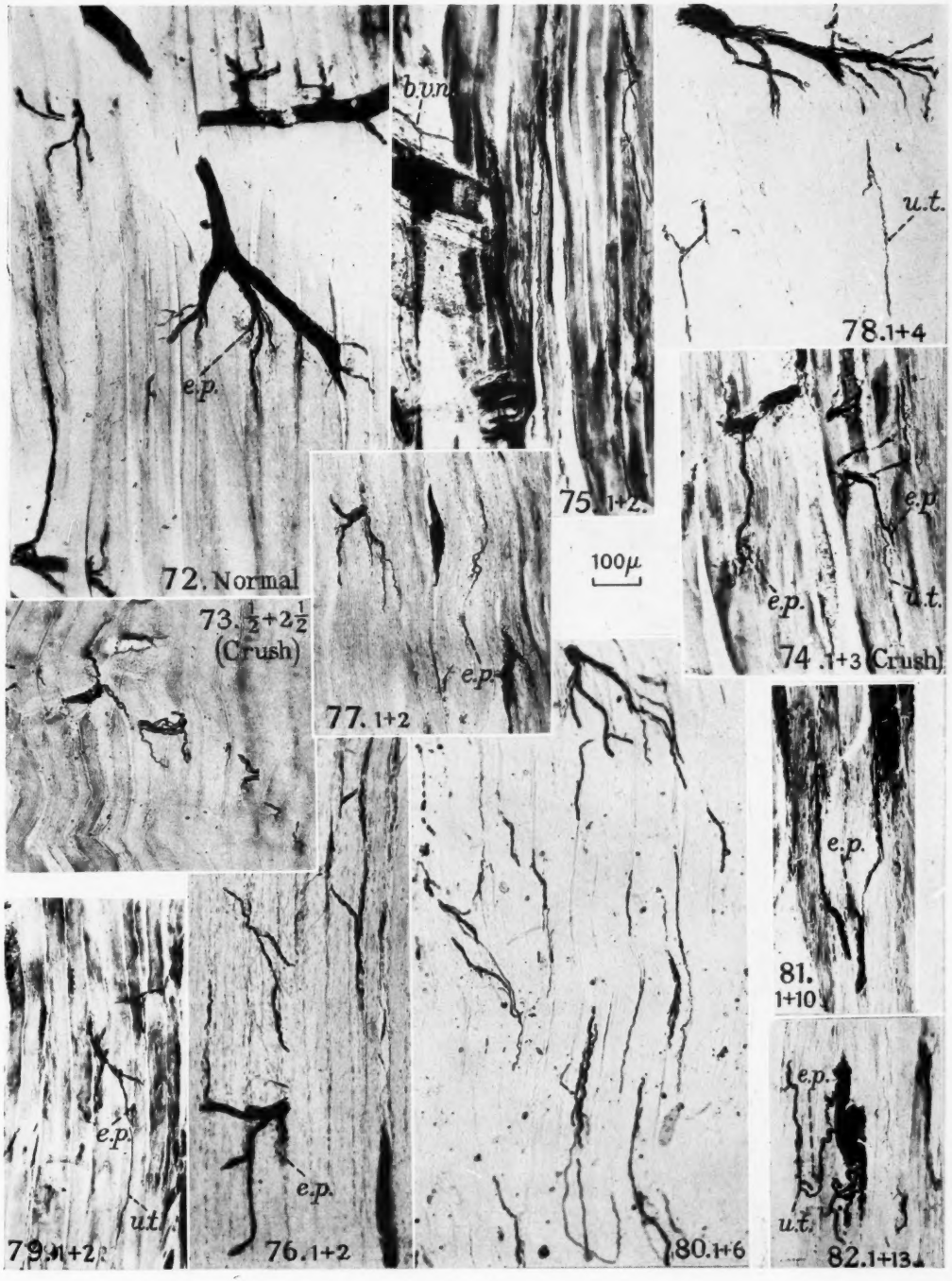
Figs. 53–62

Delayed suture. Times in months.



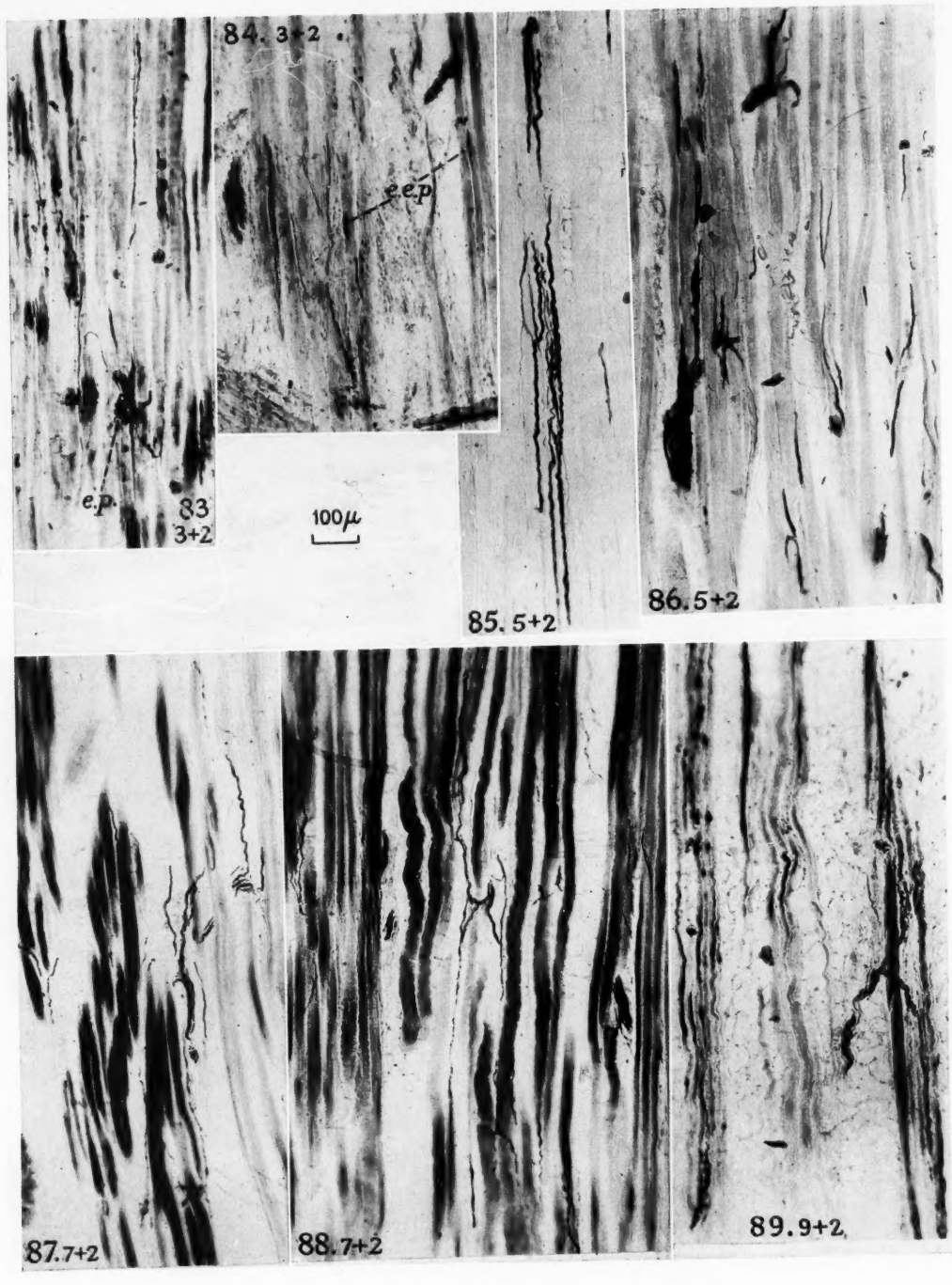
Figs. 63–71

Normal, crushed nerves, immediate suture. Times in months.



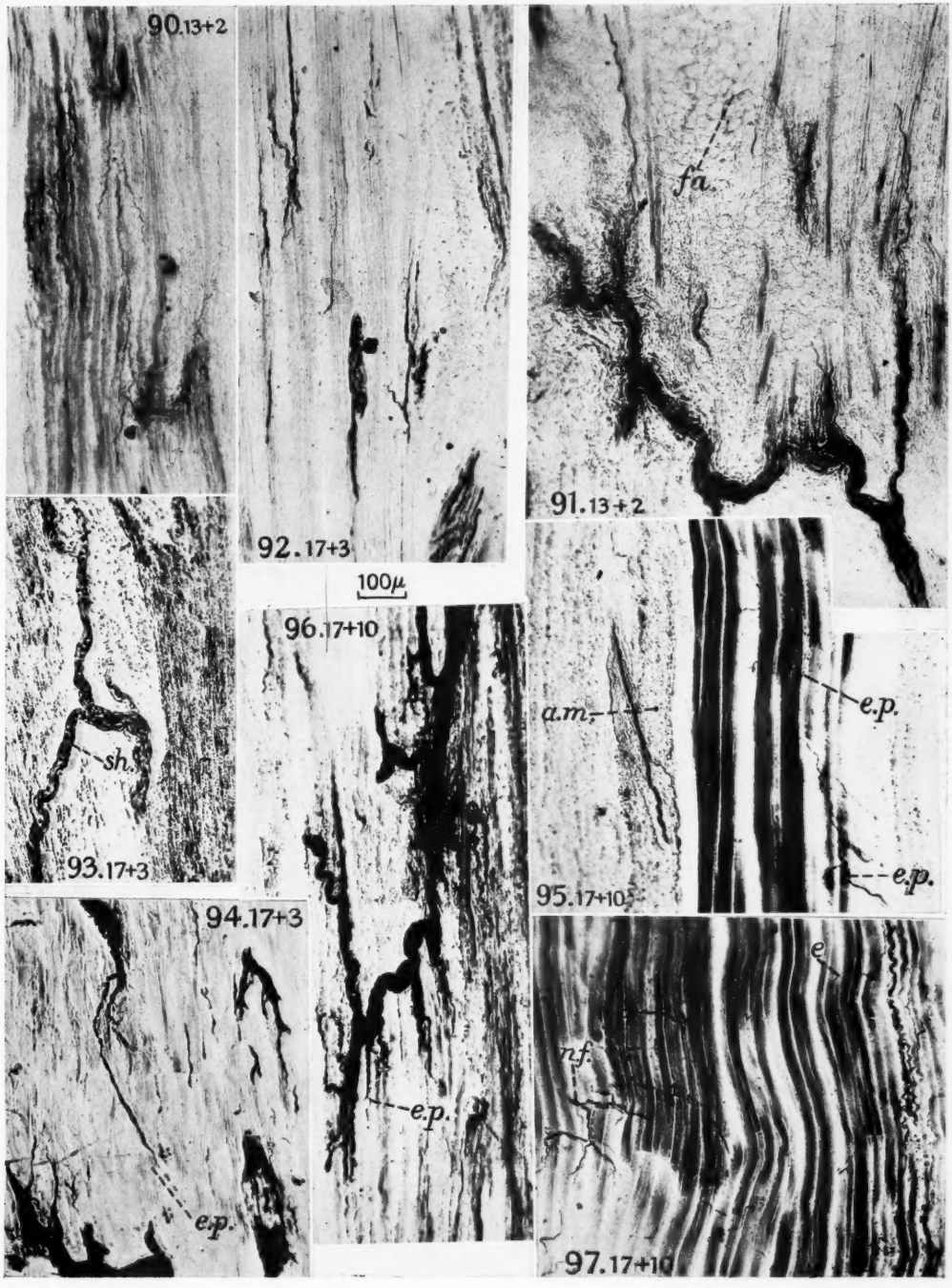
Figs. 72–82

Delayed suture. Times in months.



Figs. 83–89

Delayed suture. Time in months.



Figs. 90–97

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Fig. 67. Nerve fibre running among atrophic muscle fibres and giving off branches to make contact with them. The sarcoplasm has become differentiated around the terminal branch. (426*q* 1.)

Fig. 68. Muscle fibre remaining very atrophic long after return of fibres. Large areas of unstriated sarcoplasm are present and can hardly all be old end-plates. (426*p* 1.)

Fig. 69. Surviving end-plate projecting at side of fibre. A new nerve fibre approaches it. (426*p* 1.)

Fig. 70. Muscle fibres remaining very atrophic some time after return of fibres. (426*p* 7.)

Fig. 71. Fully formed new end-plates after long period of atrophy and long period of recovery. (452*b* 1.)

PLATES 7–9. Series to illustrate results of re-innervation after progressively increased periods of atrophy. All figures at the same magnification (75 ×). Time of atrophy and of re-innervation given after each figure number *in months*.

PLATE 7

Fig. 72. Normal muscle showing end-plates close to emergence of fibres from bundles. The one rather long fibre is quite exceptional. (732*c* 8.)

Fig. 73. After crushing nerve close to muscle the normal pattern of terminal endings is regenerated. (695*a* 1.)

Fig. 74. After crushing at a long distance from the muscle many of the new axons form ultra-terminal fibres. (702*e* 4.)

Figs. 75, 76. After immediate suture many fibres running for long distances between the muscle fibres appear. (650*c* 3, 708*e* 4.)

Figs. 77, 78. The pattern regenerated after immediate suture may be close to normal. (426*r* 1, 762*f* 4.)

Fig. 79. Ultraterminal fibres after simple suture. (650*c* 5.)

Fig. 80. The general pattern of fibres running for long distances remains even 6 months after return of fibres (671*f* 1.)

Figs. 81, 82. Ultraterminal fibres remain even 10 and 13 months after re-innervation of the muscle. (452*a* 2, 569*a* 1.)

PLATE 8

Figs. 83, 84. With 3 months of atrophy the condition is not very different from that of the simple suture. End-plates remain but often are not innervated. (650*e* 6 and 5.)

Figs. 85, 86. With 5 months of atrophy the longitudinal plexuses become very marked. (680*a* 2 and 1.)

Figs. 87, 88. With 7 months before return of fibres atrophy becomes very profound and nerve fibres run for long distances without contact with muscle fibres. (597*a* 3 and 1.)

Fig. 89. With 9 months' atrophy some muscle fibres are reduced to threads and contact with nerve fibres becomes still more difficult. (581*a* 10.)

PLATE 9

Figs. 90, 91. 13 months of atrophy. Some nerve fibres make loops *across* the muscle fibres, a condition becoming more and more frequent at later times. (P 10*g* 9 and 10.)

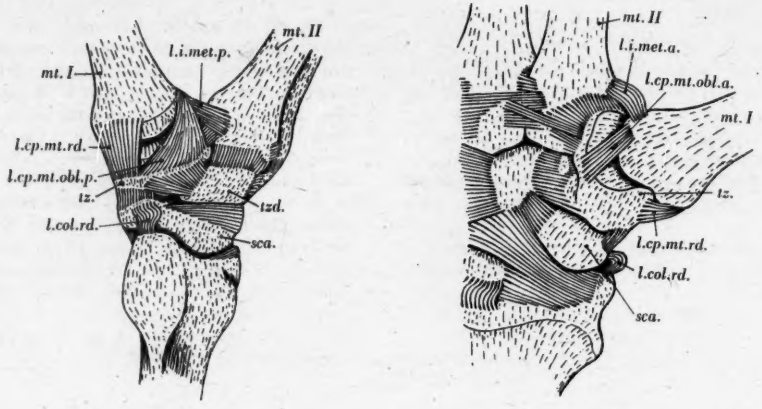
Figs. 92–94. Extreme atrophy after 17 months of denervation. Nerve fibres only seldom make contact with muscle fibres (fig. 92); they run in much thickened connective tissue sheaths (fig. 93); nevertheless, a pattern not unlike the normal may be restored (fig. 94). (426*p* 4, *p* 8 and 9.)

Figs. 95–97. Imperfect recovery even after long period of re-innervation of very atrophic muscles. New end-plates are formed, but in large portions of the muscle the nerve fibres have been unable to promote recovery of the muscle fibres (fig. 95). Nevertheless, in part the pattern of innervation may approach the normal (fig. 96). But single nerve fibres branch repeatedly as they run across muscle fibres (fig. 97). (452*b* 14 and 16.)

THE MECHANISM OF ROTATION AT THE FIRST CARPO-METACARPAL JOINT

By R. WHEELER HAINES, *Department of Anatomy, St Thomas's Hospital Medical School*

The various movements involved in opposition and reposition of the thumb have been carefully studied by several anatomists, and in particular by du Bois-Reymond (1895, 1896 *a*, *b*), who has given a detailed account of the joints involved and a full mathematical analysis of the mechanism of the saddle-joint. We shall follow this author in describing opposition as a combination of movements of the carpo-metacarpal, metacarpo-phalangeal and inter-phalangeal joints, but particular attention will be paid to the mechanism of rotation of the metacarpal bone about its own long axis.



(*a*) (*b*)

Fig. 1. Carpo-metacarpal joint of the thumb (*a*) in dorsal and radial view, and (*b*) in volar view.

Key to lettering: *l.col.rd.* radial collateral ligament of wrist-joint. *l.cp.mt.obl.a.* anterior oblique ligament of carpo-metacarpal joint. *l.cp.mt.obl.p.* posterior oblique ligament of carpo-metacarpal joint. *l.cp.mt.rd.* radial ligament of carpo-metacarpal joint. *l.i.met.a.* anterior inter-metacarpal ligament. *l.i.met.p.* posterior inter-metacarpal ligament. *mt.* metacarpal. *sca.* scaphoid. *tz.* trapezium. *tzd.* trapezoid.

If the thumb is circumducted the distal end of the metacarpal is found to move through a path which approximates to a circle, and a central position of the metacarpal can then be taken as a starting point for the description of the movements. The use of the central position as a starting point, rather than the anatomical position, greatly simplifies the account of the movements. These movements are, following the ordinary English usage, extension in the radial direction, flexion in the ulnar direction, abduction in the volar direction and adduction in the dorsal direction. At the starting point the central parts of the joint surfaces on the trapezium and metacarpal are in contact with each other, while the more peripheral parts are separated from each other by a slight gap occupied partly by synovial fluid and partly by fibro-fatty synovial folds which are well developed in this joint. As each of the four movements is carried out one surface rolls on the other so that the area of contact passes nearer the periphery of the joint, while with the rolling there is combined a very limited gliding movement (du Bois-Reymond, 1895). The movements of flexion and extension occur approximately in the plane passing through the greatest concavity of the metacarpal and the corresponding convexity of the trapezium; the movements of abduction and adduction in the plane of the greatest concavity of the trapezium.

Following his mathematical analysis of the saddle-joint du Bois-Reymond likened the articular surfaces of the joint to the region of contact of two links of a chain, so that the area of contact must necessarily be restricted, and some degree of rotation must be present. This rotation can be demonstrated, following the method of Fick (1911) and others, by using the basal phalanx as an indicator of the movement of the metacarpal bone, but care must be exercised in this use of the phalanx, for abduction, adduction and rotation,

as well as flexion and extension, may occur at the metacarpo-phalangeal joint and give a false impression of movement of the metacarpal bone. These phalangeal movements have been described in detail by du Bois-Reymond (1896*b*), and I am able to confirm his findings, though their existence was denied later by Fick. When the phalanx is fully flexed the angle it makes with the metacarpal varies greatly in different individuals, but the phalanx always faces the ulnar direction so that the plane of flexion corresponds closely to the plane of greatest concavity of the metacarpal bone at the carpo-metacarpal joint.

If, with the muscles relaxed, the phalanx is used as a lever to rotate the metacarpal bone about its own long axis, or alternatively, if the metacarpal is held rigidly while the trapezium is moved by pronating and supinating the forearm (an action which most individuals find rather easier to carry out), it can be demonstrated that passive rotation of about 45° is permitted at the carpo-metacarpal joint without the use of undue force. If the carpo-metacarpal joint is but slightly flexed and extended the whole movement is a simple one through the plane of greatest concavity of the metacarpal. But towards the end of flexion there is a sudden swinging of the phalanx towards the palm, indicating an axial rotation (in the sense of pronation) of the metacarpal bone through about 30°, and at the same time the capability of passive rotation disappears, so that when the metacarpal is forced into the fully flexed position it is at the same time fully rotated. Any attempt to undo the rotation leads immediately to some degree of extension. Similarly, towards the end of extension there is usually an axial rotation of the metacarpal in the opposite direction, but of about 15° only. So we find that the metacarpal can be rotated passively while it is near the centre of its range of movement, but that towards either limit in the plane discussed it undergoes a forced axial rotation in one direction or the other. Du Bois-Reymond (1896*b*) has worked out the amounts of this rotation associated with the whole perimeter of circumduction, but these movements are more difficult to understand than the simple flexion and extension considered above, since they require for their description a use of spherical trigonometry.

It has been seen that axial rotation occurs during passive movement with the muscles relaxed, which suggests that the causation is not directly muscular. A ligamentous preparation confirms this. When the metacarpal is near its central position in such a preparation all ligaments are slack and the joint allows a wide range of axial rotation, but towards the extremes of flexion and extension the metacarpal rotates just as in the living hand. On removing the rather dense connective tissue surrounding the joint three strong ligaments are seen, for which I propose the names radial, anterior oblique and posterior oblique carpo-metacarpal ligaments. The first is a broad band from the radial side of the trapezium to the base of the metacarpal, the other two arise from the anterior and posterior surfaces of the trapezium, and converge to be inserted near each other on a bony elevation of the ulnar side of the base of the metacarpal. A weak anterior and a strong posterior inter-metacarpal ligament unite the first and second metacarpal bones. Besides these there may be some accessory bands, but I have found them inconstant.

When the joint is near its mid-position all the ligaments are slack, so that circumduction and axial rotation in either direction are allowed, but when the joint is flexed the posterior oblique ligament, assisted by the posterior intermetacarpal ligament, becomes taut, and pulling on the ulnar side of the base of the metacarpal bone forces it to rotate. Similarly, when the joint is extended the anterior oblique ligament causes axial rotation in the opposite direction. Cutting the ligaments destroys the mechanism. Abduction also leads to a tightening of the posterior oblique ligament and to an enforced rotation. In the living hand the muscles are of course responsible for the movements, but the axial rotation is determined by the ligamentous arrangement.

That these important ligaments have remained so long unknown, in spite of the interest of clinicians and anatomists in the movements of opposition, is, I believe, primarily due to the lack of a clear understanding of the nature of ligamentous tissue. The quadrate radio-ulnar ligament and the capsules of most joints are composed of dense connective tissue, while, on the other hand, the cruciate or collateral ligaments of the knee or the oblique ligaments of the carpo-metacarpal joint of the thumb are built essentially of straight fibre bundles giving a much less extensible tissue. It may well be that these two kinds of tissue, the one similar to fascia or adventitial coats, and the other to tendons or aponeuroses, may be found to grade into one another, but for practical purposes they are quite distinct: the one is teased away by dissection while the other adopts a polished appearance as the fibrous tissue surrounding it is removed and the direction of the fibre bundles becomes clear. In the carpo-metacarpal joint of the thumb Fick's classical drawing is of the dense connective tissue surrounding the joint, not of the straight-fibred ligaments, but it is drawn and coloured as if it were of the same nature as the inter-metacarpal ligaments.

Moreover, our conception of the function of ligaments may need revision. It is usual to describe many ligaments as preventing excessive movement, and to think of them as slack throughout the greater part of the range of movement, but as

tightening suddenly as the end of the range is approached and so limiting the movement. The anterior and posterior cruciate ligaments of the knee joint, for instance, have been said to limit extension and flexion respectively, and the anterior and posterior radio-carpal ligaments have been assigned similar functions. On the other hand some ligaments, notably the annular ligament of the elbow and the transverse ligament of the atlas, have always been considered as guiding rather than as limiting movements and as remaining taut over a long range of movement rather than as suddenly becoming taut as the limits of movement are reached. At the same time the former conception has often been questioned, notably by Partridge (1924), who believed the ligaments to be incapable of taking the sudden strains which would be involved in checking movements, so that she supposed them to be rather of the nature of sensory organs for the reflex activity of the muscles. Even the importance of ligaments in the mechanism of the body has increasingly been questioned. For instance, in the support of the arches of the foot most authors have followed Keith (1929) in ascribing the greater part of the work to muscles.

Yet Jones (1941) has shown recently that even when the arches of the foot are subjected to great loads the muscles may be quite relaxed. The truth seems to be that many ligaments have the dual function of restraining and of guiding movements; for example, the anterior and posterior cruciate ligaments of the knee are now known to act throughout the movement of the joint, so as to regulate the relative position of the femur and tibia, though they also help to limit extension (Haines, 1941). Similarly, the ligaments of the carpo-metacarpal joint of the thumb seem to be arranged so as to determine an axial rotation of the metacarpal bone at the end of the movements of flexion and extension, though they act as limiting agents also.

It seems probable that a careful examination of the ligaments of other joints will show that they have guiding actions which modify the direction of the forces exerted by the muscles.

SUMMARY

1. Certain hitherto unrecognized ligaments associated with the first carpo-metacarpal articulation are described.

2. It is suggested that their function is mainly a guiding one, and that they are responsible for the axial rotation of the first metacarpal bone which takes place at the end of the movements of flexion and extension at the first carpo-metacarpal joint.

3. It is suggested that the ligaments of some other joints also have a regulating action on the bones concerned.

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THE ANATOMICAL BASIS OF CORTICO-STRIATE CONNEXIONS

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Recently the striatum (a term which is here taken to include the caudate nucleus and the putamen) has attracted fresh interest by reason of the remarkable discovery of the cortical suppression areas in the monkey and the cat brain. In their paper on 'Physiological neuronography of the cortico-striatal connexions' (1942), Dusser de Barenne, Garol & McCulloch write: 'In 1937 we were engaged in mapping the functional organization of the sensory cortex of the monkey and ran into difficulty with a narrow strip of cortex at the anterior margin of area 4. When this narrow strip is stimulated electrically, chemically or mechanically, there follows, after a latency of minutes, a transient diminution of electrical action, first seen in area 4 and later observed to sweep across the entire sensory cortex and even beyond it.' This narrow strip of cortex was called area 4S. About the same time, Hines (1937) had found that stimulation of this area 4S caused sudden cessation of spontaneous activity, and relaxation of existing muscular contractions. Dusser de Barenne *et al.* carried out further ingenious and detailed experiments in which, besides the area 4S, further suppression areas (8S, 2S and 10S) were found. They concluded from their observations on the changes in electrical potentials which occurred in the striatum during stimulation of the suppression areas that the resulting cessation of activity in the cortex is due to a discharge from the caudate nucleus and the putamen. Thus they demonstrated by electro-physiological methods an evident connexion between the suppression areas and the striatum. They suggested that the further pathway from the striatum back to the cortex has one or more relay stations, which may be situated in the thalamus. These results, first obtained in the monkey brain, were confirmed by similar experiments on the cat brain by Garol (1942) (Text-fig. 1). Garol gave a new description of the motor area in this animal, mapped out carefully the sensory areas of the brain, and found the suppression areas 8S, 3S, 2S and 19S. These four areas, two (3S, 2S) lying within the sensory cortex and two (8S, 19S) lying outside it, have been shown to effect suppression of motor responses. Local strychninization produces a temporary suppression of the electrical activity, which sweeps over the entire convexity of the hemisphere.

From the anatomical point of view the question of cortico-striate connexions has been widely discussed for many years. More detailed reference to the anatomical literature on this subject appears in the discussion, but it may be stated here that one of the most recent studies on this question, by Verhaart & Kennard (1940), has not confirmed the existence of any definite cortico-striate connexions, and this seems at first sight to be in serious conflict with the physiological data mentioned above. We now describe experiments which we have ourselves carried out in an attempt to obtain further information on the much disputed existence of direct fibre connexions between cerebral cortex and striatum.

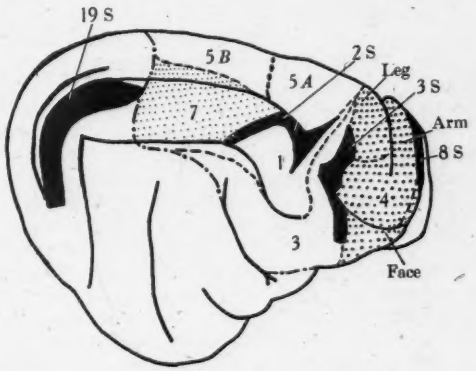


Fig. 1. The functional organization of the cat's cortex, re-drawn after Garol (1942). The suppression areas are shaded in black.

MATERIAL AND METHODS

Mature rabbits and cats were used for the present experiments. For the study of normal material the brains of rabbits and of foetal and mature cats were used. For the experimental cat material we are indebted to Prof. Liddell, who allowed us to study brains in which lesions had been made during the course of his study of the pyramidal system. The rabbits used were anaesthetized with nembutal and, after opening the skull, localized lesions of the cortex were made, either with a knife or with a hot needle. Lesions in the caudate nucleus were made but cutting with a fine ophthalmic knife passed through the cortex, the site of each lesion having been previously determined by a careful topographical study in a dead animal. The animals recovered in a few hours after operation. The cats, operated on in the Physiological Laboratory by

Prof. Liddell, were anaesthetized with nembutal. The operations were effected by opening the skull and cutting a window in the dura mater. (The orientation of the cat brain is comparatively easy because of the relatively constant arrangement of the cortical sulci and gyri.) Cortical and nuclear lesions were made with a sharp knife. In some instances the motor cortex was first mapped out by stimulation with sterilized electrodes. The recovery of the animals was uneventful.

Technique

(*a*) *Marchi method.* For staining the degenerated myelin the Swank-Davenport modification of the Marchi technique was used. This technical process was further modified for work on frozen material as follows: the brain is carefully removed and fixed in 10 % formalin (neutral) for 48 hr.: it is then cut into slices of about 3–5 mm. thickness. The slices are washed for 5–10 min. in a 1 % $KClO_3$ solution; they are then transferred into the Marchi solution recommended by Swank and Davenport, viz. 20 c.c. of osmic acid 1 %, 60 c.c. of $KClO_3$ 1 %, 20 c.c. of formalin 37 %, 1 c.c. of glacial acetic acid. The slices remain in an appropriate quantity of this solution for about 10–12 days, and are afterwards washed in tap water for 2 hr. They are cut with the freezing microtome to a thickness of 60–90μ. The frozen sections are put into distilled water, thence into 96 % alcohol for half an hour; they are then cleared in creosote and mounted with Canada balsam, each under a cover-glass.

(*b*) *Modified Spielmeyer method.* For the study of normal myelinated fibres the following modification of Spielmeyer's method proved very useful. The removed brain is fixed for 10 days or more in formalin and afterwards washed for several hours in tap water. Frozen sections of 30μ thickness are cut and placed in 5 % iron alum for 12 hr. The sections are then washed quickly in distilled water and immersed in 50 % alcohol for 10 min., being agitated continuously the while. They are then transferred into an old solution of haematoxylin (5 parts of a well-ripened 10 % solution of haematoxylin in alcohol and 100 parts of distilled water) and are stained for 5 hr. or more. They are next washed quickly in distilled water and differentially stained in 2 % iron alum until the grey matter becomes just visible. Further differential staining is effected in a 5 % oxalic acid solution until the sections turn yellowish brown, whereupon they are quickly transferred into a weak solution of ammonia water, wherein they turn blue. Lastly, they are placed in tap water for 1 hr. and are then mounted in the usual way.

(*c*) *Silver method.* After technical trials for more than two years the following silver method proved to be the most satisfactory for staining nerve fibres with their finest terminal branches. The brain is fixed for 4 days or more in 10 % formalin. Slices are then washed overnight in running water and brought into distilled water for 2 hr. Sections are cut on a freezing microtome to a thickness of about 15–20μ. They are washed again in distilled water for 1 hr., and then transferred into a mixture of 20 c.c. of a 20 % silver solution and 50 c.c. of absolute alcohol, wherein they stain yellowish brown. Afterwards they are washed in a 10 % formalin solution and transferred into a solution of silver nitrate to which has been added ammonia and 10 drops of absolute alcohol. In this solution the sections stain for a brief period only, and are reduced in a 1–10 % formalin solution, the strength of which depends on factors such as the species of animal employed (i.e. rabbit, cat, or monkey) and the length of time of previous fixation. They become deeply stained, and are then placed in distilled water and briefly washed in hypo solution. After this procedure they are washed in distilled water for half an hour and are then mounted in the usual way.

NORMAL HISTOLOGY OF THE CAUDATE NUCLEUS

We shall consider only those parts of the normal histology which are of importance for the present investigation. The caudate nucleus is bordered laterally by the heavily myelinated fibres of the internal capsule, and dorsally by the much finer myelinated fibres of the fasciculus subcallosus or stratum subcallosum, from which a small number of thinly myelinated fibres descend medially and ventrally to form a fine layer of nerve fibres beneath the ependyma covering the medial surface of the nucleus. In normal myelin preparations some myelinated fibres from the stratum subcallosum give the appearance of penetrating into the superficial zone of the caudate nucleus. This has been illustrated by Papez (1942). Using a myelin strain, preferably on frozen sections (which allow slow and individual differentiation of the stain), it is possible to see inside the caudate nucleus a fair number of very finely myelinated fibres, loosely arranged among the cells. Followed for some distance, these fibres are found to collect at fairly regular intervals into small bundles of 20–30 fibres, which pierce the internal capsule in order to descend in a ventral direction through the putamen. This fibre system is referred to as 'radiate bundles' by previous authors (Pl. 1, fig. 3).

The most impressive feature in a silver-impregnated section of the caudate nucleus is the density and complexity of the non-myelinated fibre plexus (Pl. 1, fig. 2). This plexus attains its greatest density immediately around the cells, forming secondary pericellular plexuses (Pl. 2, fig. 1). In examining these sections, after their counterstaining with

toluidin blue, the impression is gained that the plexus is composed of dendrites and terminal arborizing axons. Counterstaining with toluidin blue facilitates the analysis of the plexus, because all cells are stained and those fibres of the plexus which are dendrites can be recognized as such since their connexion with the cell body can be established. Occasionally some of the terminal axons seem to end in a little knob, but on the whole their precise mode of termination is extremely difficult to demonstrate. Turner (1903) described such terminal boutons on the cells of the caudate nucleus. On the other hand, Bielschowsky (1919) was unable to detect these end-feet, though he found many of them around the large cells of the pallidum. We must therefore assume, until we acquire further knowledge, that the morphological substratum of the synapse in the striatum is predominantly represented by a pericellular plexus, very similar to the pericellular plexuses of the cerebral cortex, in which terminal boutons, if present, are very few in number. The cell types of the striatum have been studied in detail by Cajal (1909) and Bielschowsky (1919).

A comparison of sections stained for myelin with those stained by silver shows, in the latter, fibres passing from the internal capsule towards the caudate nucleus and ramifying among its cells. These fibres are collaterals from fibres occupying the internal capsule (Pl. 1, fig. 1). They branch off at a right angle from the main descending fibres and turn towards the caudate nucleus, crossing the radiate bundles emerging therefrom. These collateral fibres always run singly and are never grouped in bundles like the strio-fugal fibres.

EXPERIMENTAL OBSERVATIONS ON THE RABBIT BRAIN

In numerous experiments on the rabbit brain, in which we repeated the extirpation of cortical areas previously investigated by Coenen (1927), we removed the following regions: area 4, 1–3, 5–7, 13–16, 21 and 22, as determined by reference to the atlas of the rabbit brain by Winkler & Potter (1911). The animals were allowed to survive for four weeks and the material was stained by the Marchi method already described. It was impossible, however, to confirm Coenen's statement that extensive connexions exist between the areas centralis and temporalis and the caudate nucleus. In all our experiments with lesions of area 4, and of the frontal, parietal and temporal areas, the caudate nucleus showed no degeneration of its myelin content (Pl. 1, fig. 5). On the contrary, lesions of the caudate nucleus itself proved that the radiate bundles are composed entirely of caudato-fugal fibres. As we had already found in sections of normal preparations stained for myelin that at least the great majority of the myelinated fibres in the caudate nucleus are efferent, the negative evidence of the Marchi method was not surprising. The conclusion was thus reached that all the myelinated axons belong to the efferent system of the caudate nucleus and that afferent connexions from the cortex, if present, must be sought in non-myelinated fibres.

EXPERIMENTAL OBSERVATIONS ON THE CAT BRAIN

In the brains of three cats, the cortical areas removed included, besides the motor area and some parts of the sensory area, the suppression areas 2S, 3S and 8S (Garol, 1942). We stained the sections with the same silver method which in normal preparations revealed a dense non-myelinated plexus. Since the results of all three experiments are identical, we describe the collective observations. The animals were allowed to survive for four weeks. The brains were fixed in formalin, which allows some slices to be stained for degenerated myelin and others by the silver method. Sections stained by the Marchi method showed no degenerated myelinated tracts extending from the injured cortex into the caudate nucleus. But sections stained by the silver method gave different information. Numerous degenerated axons could be traced, leaving the internal capsule to enter the caudate nucleus (Pl. 1, fig. 4). These afferent fibres were swollen and fragmented. The point of ramification, whereat the main fibre breaks up into preterminal branches, was greatly swollen. The preterminals were fragmented and some of the fragments were thickened (Pl. 2, fig. 2). The preterminals came into close contact with the cell body and gradually continued as free terminals. These terminal arborizations were clearly in a state of degeneration, having a fine beaded appearance; some of these thickened knobs possibly represent a stage in the degeneration of true terminal boutons (Pl. 2, fig. 3). Our preparations indicate that each main afferent fibre is in contact with numerous cells and that neighbouring fibres overlap one another extensively.

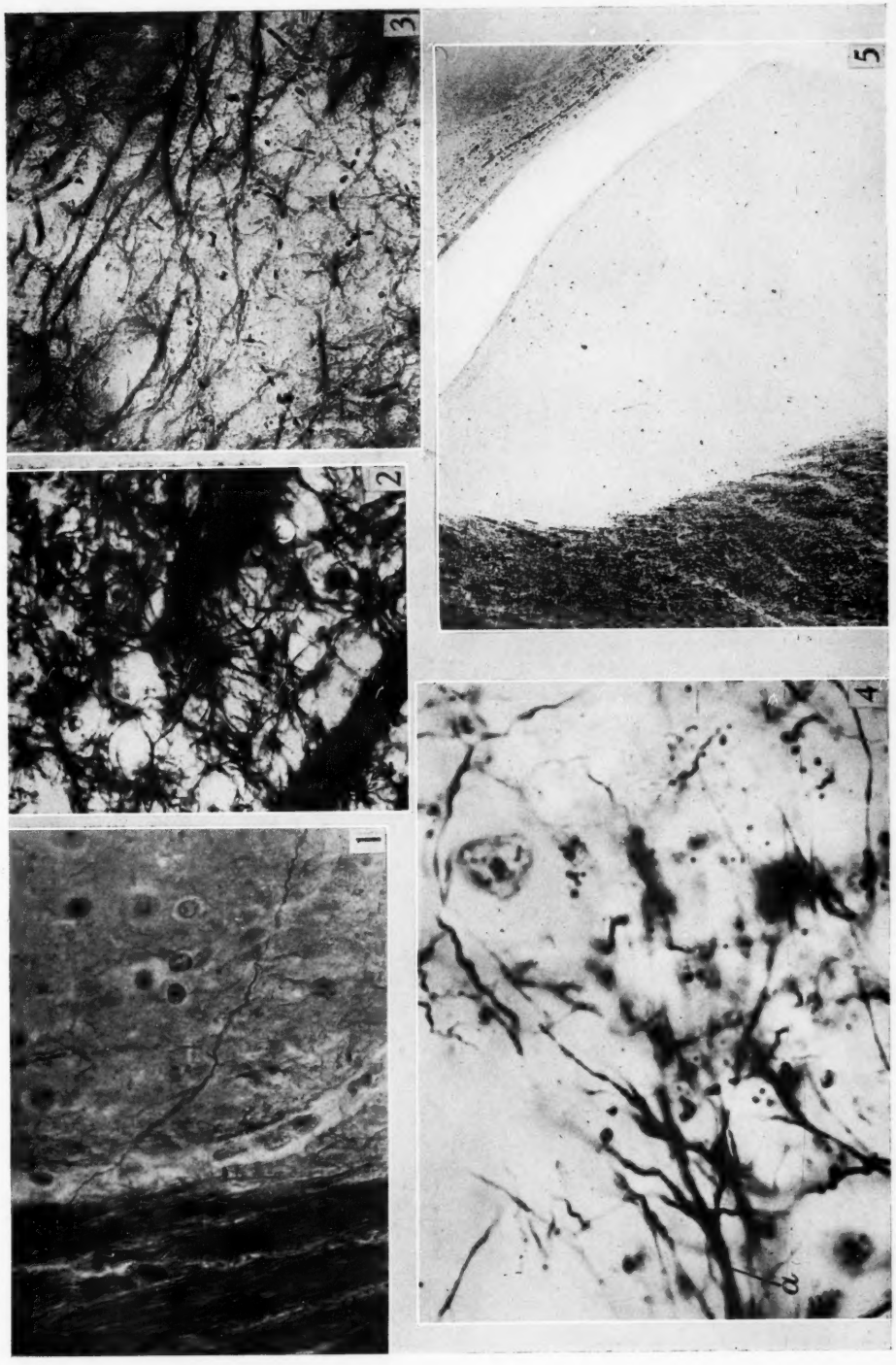
In one experiment the leg motor area only had been removed. In this particular animal the caudate nucleus was free of degenerated non-myelinated fibres, which is in accordance with the observations by Dusser de Barenne and McCulloch, whose electrophysiological observations showed that the motor cortex has no direct connexion with the caudate nucleus.

DISCUSSION

The most important experimental paper on the corpus striatum up to 1940 was that by Wilson (1913, 1914). His experiments on the monkey brain

dealt mainly with lesions in the putamen and globus pallidus. No lesions were made in the caudate nucleus itself. He divided the fibre system of the striatum into four groups: (1) fibres (internuncial) arising and ending within the corpus striatum, (2) fibres (strio-fugal) arising in the corpus striatum and ending elsewhere, (3) fibres (strio-petal) arising elsewhere and ending in the corpus striatum, (4) fibres (fibres of passage) passing through the striatum but arising and ending elsewhere. Of the internuncial fibres the most important are those from the putamen to the pallidum and those passing between the caudate nucleus and the putamen. The second group has the following divisions: strio-thalamic fibres, strio-subthalamic fibres, and strio-fugal fibres to the internal capsule or cerebral peduncle. The third group is, for the purposes of our present investigation, the most important. Wilson described in this group the following fibre tracts—thalamo-striate and subthalamo-striate connexions. Considering the question of a cortico-striate relation, he was of the opinion that the corpus striatum is independent of the cerebral cortex. His experiments adduced no evidence for cortical fibres entering the caudate nucleus, nor did he find evidence of Cajal's collaterals from the cortico-spinal system arborizing among the cells of the striatum. A similar view was taken by Pollak (1922), who, in a comprehensive survey of the literature up to that date, concluded that the evidence for direct cortico-striate connexions was so meagre as to be negligible. He supported this view by reference to the clinical and experimental findings of Wernicke (1884), Probst (1899), Dejerine (1901), Spiegel (1907), Gruenstein (1911, 1924) and C. & O. Vogt (1919). Pollak suggested that the principal afferent system to the striatum is from the thalamus; on the other hand he did not deny the existence of the cortical collaterals demonstrated by Cajal, whose function he considered to be of an associative nature. In favour of direct cortical connexions were the observations of v. Economo (1910). Wallenberg (1925), however, was unable in his experiments with the Marchi method to find such connexions, but v. Monakow (1924) reported a distinct atrophy of the corpus striatum after extirpation of the cortex in a dog. Minkowsky (1923) reported fibres running from the precentral gyrus to the caudate nucleus, and from the frontal lobe to the caudate nucleus and pallidum. Coenen (1927), using the Marchi method in rabbit experiments, concluded that connexions exist between the areas centralis and temporalis and the caudate nucleus through the medium of fibres running through the fasciculus subcallosus and to a certain extent along the dorsal edge of the internal capsule. Igarasi (1939–40) was of opinion that the caudate nucleus is connected with the front part of the cerebral hemisphere: he observed fibres from the stratum subcallosum entering the caudate nucleus, but these were of extremely fine calibre and were few in number. From our brief survey of the anatomical literature it becomes apparent that there has been a prolonged search for cortico-striate connexions, but most of the observers have been able to demonstrate but relatively few fibres establishing such a pathway. The course of this pathway, as agreed by most research workers, is the fasciculus subcallosus, or stratum subcallosum. Coenen (1927) believed that his sections also showed fibres entering *via* the internal capsule, but, judging from his photographs of Marchi preparations, it is very probable that he was misled by pseudo-Marchi granules. Mettler (1942), in his careful analysis of the fasciculus subcallosus, based on numerous experiments, supported the findings of previous authors, namely, that the fasciculus carries fibres to the caudate nucleus. According to the results of Verhaart and Kennard, however, the subcallosal fasciculus contains only a small number of very fine degenerated fibres following cortical lesions, but none could be seen entering the caudate nucleus and the nucleus itself was found to be clear. In our opinion the fasciculus subcallosus certainly carries no fibres from the suppression areas, or from the neighbouring areas of the cortex, to the caudate nucleus. On the other hand, we do not wish to exclude the possibility that some part of the frontal area of the cortex may contribute to the fasciculus, but, if this be so, the fibres must lose their myelin sheaths on entering the caudate nucleus, for any extensive degeneration of intracaudate fibres is scarcely perceptible in Marchi sections of the rabbit brain in which the frontal cortex has been injured. Mettler (1942) and Papez (1942), in their contributions to the book on the *Basal Ganglia*, give a most valuable survey of all the literature on the connexions of the striatum.

Since we had been unable in our Marchi experiments to demonstrate direct cortico-caudate connexions in the cat and rabbit, we came to the conclusion that, if these exist, they must consist of fibres which are unmyelinated, or at least of fibres which lose their myelin sheath on entering the nucleus. Accordingly, we sought for evidence of terminal axonal degeneration by the application of a silver impregnation technique, after making circumscribed cortical lesions which included the suppression areas, and in this search we were successful. A definite degeneration of the non-myelinated pericellular plexus seen in normal preparations was evident. The degenerative changes in the preterminal and terminal fibres were quite similar to those previously demonstrated in the lateral geniculate body of the monkey (Glees & Le Gros Clark, 1941), cat (Glees, 1941) and rabbit (Glees, 1942). The degeneration of the cortical fibres running into the substance of the caudate nucleus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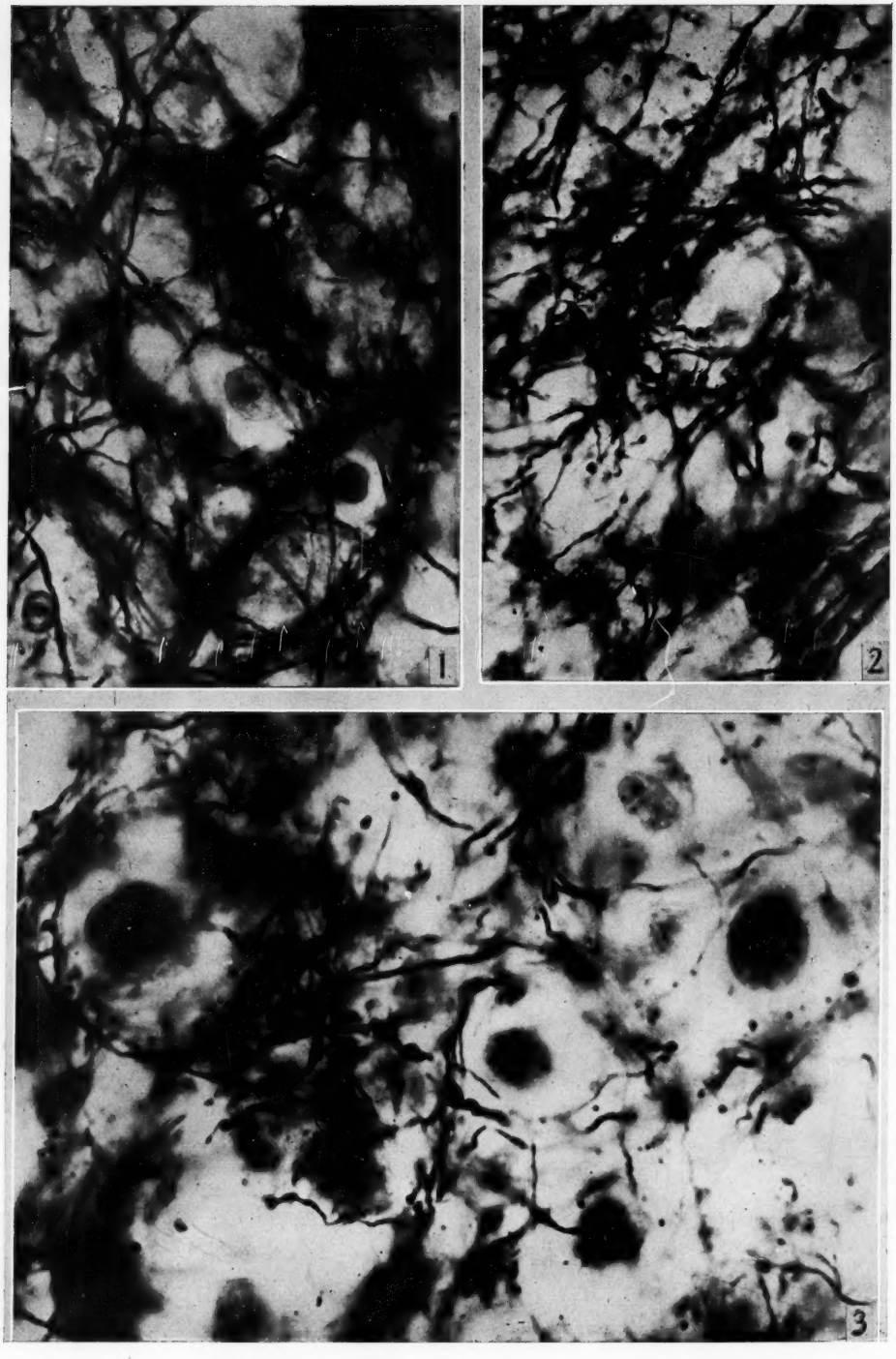

P. GLEES—THE ANATOMICAL BASIS OF CORTICO-STRIATE CONNEXIONS

could be followed in continuity from the internal capsule, the fibres turning inwards obliquely or at a right angle. They lose their myelin sheaths on entering the nucleus and for the most part run singly. In the degenerated state, the fibres break up into small or large fragments. At the branching point of the afferent fibres a swelling usually occurs, and the preterminal fibres, originating from the swelling, are beaded or fragmented and are slightly swollen. The terminal fibres break up into very fine, small and deeply staining fragments, which are in the closest relationship with the cell surfaces. These terminal fibres, forming under normal conditions the pericellular plexuses, establish synaptic connexions with the cell body. In degeneration, some cells are observed to be covered with minute fine, black granules and tiny dots, and it is not impossible that these products of degeneration represent what in the normal state are minute terminal boutons.

SUMMARY

1. The areas of suppression, 2S, 3S and 8S, in the cat brain are connected with the caudate nucleus by non-myelinated cortico-caudate fibres arising as collaterals from cortico-fugal fibres.

2. These cortico-caudate fibres ramify among the cells of the caudate nucleus and enter into the formation of pericellular plexuses.

3. The degeneration of these fibres after cortical lesions can be demonstrated in silver preparations as typical preterminal and terminal degenerative processes.

I wish to thank Mr W. Chesterman for his skilful technical assistance in the prosecution of this research.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Caudate nucleus of the cat. A collateral fibre is seen leaving the internal capsule on the left and entering the substance of the nucleus. Silver technique. ×350.

Fig. 2. Section of the caudate nucleus of the cat, showing the density of the pericellular plexuses. Parts of the radiate bundles are also shown. Silver technique. ×350.

Fig. 3. Section of the caudate nucleus of the rabbit, stained for myelin. It shows the myelinated axons collecting to form the radiate bundles which leave the nucleus. ×210.

Fig. 4. Silver section of the caudate nucleus of a cat after a cortical lesion in the gyrus sigmoideus anterior and posterior, including the suppression areas 8S, 3S and 2S. A cortical collateral in course of degeneration (*a*) is seen entering the nucleus and ramifying among its cells. Note the thickening of the fibres at the point of its ramification. ×800.

Fig. 5. Marchi section of a cat brain, in which the gyrus sigmoideus anterior and posterior, including the suppression areas 8S, 3S and 2S, had been removed. No Marchi degeneration is present inside the caudate nucleus. Note the degenerated fibres of the corpus callosum, at the right upper corner of the photomicrograph. ×12.

Plate 2

Fig. 1. Normal pericellular plexuses of the caudate nucleus of the cat, stained with silver. ×800.

Fig. 2. Degenerated pericellular plexuses in the caudate nucleus of a cat, in which a lesion involving the suppression areas had been made 4 weeks previously. ×800.

Fig. 3. A view at a higher magnification of the terminal degeneration of cortico-striate fibres. Note the fine beads of argentophil substance on the surface of the cells. These products of axonal degeneration are similar to the degenerative enlargements of terminal boutons seen elsewhere in the central nervous system. Such boutons, however, if present in the synaptic formations in the normal caudate nucleus, are too fine for demonstration by available technical methods. ×1600.

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF THE PRONEPHRIC DUCT IN ANURAN EMBRYOS*

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INTRODUCTION

The development of the pronephric duct has long been a matter of dispute. Field (1891) believed that in amphibians the nephric rudiment giving rise to the pronephric duct is formed *in situ* by a contribution from each segment. Brauer (1902), on the other hand, described an independent caudal growth of the nephric rudiment in Gymnophionia. Of these two conceptions, the first has been accepted by the majority of authors.

Recently, O'Connor (1938, 1939) has studied experimentally the mode of the caudal extension of the pronephric duct in *Amblystoma tigrinum* and *Triton taeniatus*. He found that when the caudal limit of the nephric rudiment is stained with Nile blue sulphate the dye can be traced in the pronephric duct cells from the stained area to the cloaca. Furthermore, if the caudal growth of the nephric rudiment is prevented by a piece of tissue grafted in the path of its extension, no pronephric duct is formed caudal to the transplant. He concluded, therefore, that the pronephric duct in these species is formed not in a segmental manner, but by its own independent caudal growth.

The present experiments were performed on anuran embryos. The results tend to confirm those obtained in Urodeles by O'Connor. The main part of the experiments, however, concerns the factors responsible for the determination of caudal extension of the nephric rudiment. It seems that there is a close functional and topographical relationship between the pronephric duct and the path along which the rudiment normally grows.

MATERIAL AND METHODS

Bufo bufo gargarizans and *Rana nigromaculata* were used in these experiments. The embryos chosen for operation varied from 6 to 10 somites when the nephric rudiment has not yet reached the cloaca. Four groups of operations were performed: (*a*) removal of the caudal end of the nephric rudiment; (*b*) resection of the nephric rudiment; (*c*) rotation of the caudal end of the rudiment through 90° or 180°; (*d*) rotation of the tissue caudal to the caudal end of the nephric rudiment through 90° or 180°. All the specimens were fixed in Bouin's solution, sectioned serially at 10μ and stained with alum cochineal.

Reconstructions were carried out by drawing the sections with the aid of a camera lucida, measuring the width of the structures as well as their distance from a mid-line and plotting these measurements to scale on squared paper. Other figures are camera lucida drawings.

EXPERIMENTAL

(*a*) *Removal of the caudal end of the nephric rudiment*

The operation was made at the stage when the nephric rudiment had reached the ventro-lateral border of somites 6–8. A square piece of tissue one somite in width, including the caudal end of the nephric rudiment, was extirpated. The gap was filled by a small piece of agar impregnated with Nile blue sulphate. The agar is not easily maintained in position, and it was usually pushed out of the body or into the gut several hours after operation.

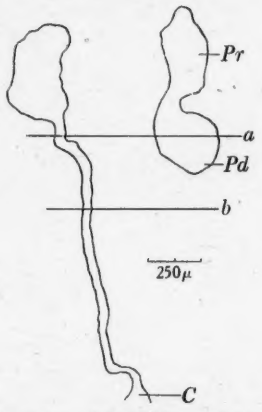

Fig. 1. Nephric system of *Bufo*. The caudal end of the left pronephric rudiment removed and the caudal growth obstructed by a piece of agar. *a*, level of Fig. 2*a*; *b*, level of Fig. 2*b*; *c*, cloaca; *Pd*, pronephric duct; *Pr*, pronephros.

In eleven cases out of eighteen the pronephric duct on the operated side ceased to grow at the region of operation (Fig. 1). It ended blindly and

* Preliminary account read at the 3rd meeting of the Chêngtu branch of the Chinese Physiological Society, 25 March 1939.

in most cases underwent a considerable dilatation (Fig. 2*a*). No pronephric duct was found immediately caudal to the region of operation (Fig. 2*b*) except in one case where at the caudal end of the embryo there existed a small short duct attached to the cloaca. Its position and histological appearance resembled those of the pronephric duct. A similar structure in *Amblystoma* has been described by O'Connor, who interpreted it as a contribution from the cloaca to the hindermost portion of the pronephric duct. All these cases demonstrate clearly that the pronephric duct caudal to the 6th somite is formed by an independent caudal growth of the nephric rudiment and not by a contribution from each individual segment.

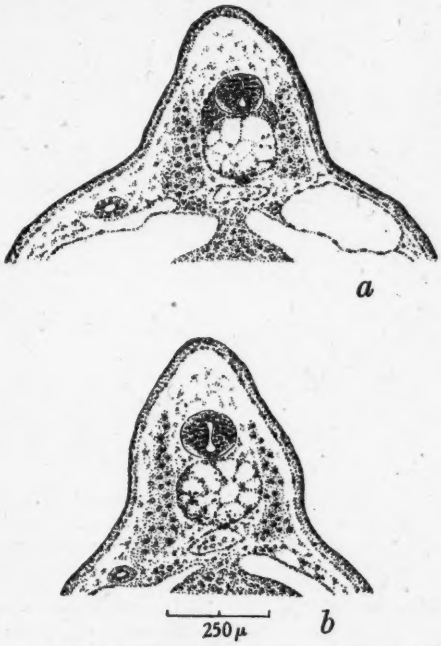

Fig. 2. *a. Bufo*, experiment of Fig. 1, showing the dilatation of the pronephric duct cranial to the region of operation. *b. Bufo*, experiment of Fig. 1, showing the absence of the pronephric duct caudal to the region of operation.

In the remaining seven cases the development of the pronephric duct was not affected by the extirpation of the caudal end of the rudiment. A pronephric duct leading from the pronephros to the cloaca was formed on the operated side. It became irregular in most cases as it came into the operated region. In one specimen the duct became dilated immediately cranial to the operated region. It then reduced its size throughout this region and recovered its normal form in the caudal segment. The interpretation may be that the portion of the nephric rudiment some distance from its caudal end has also the capacity of growth. In these cases the grafted agar was probably not well held in place and the healing of the wound followed immediately after the operation; thus the caudal growth of the cut end of the rudiment would take place normally without any obstruction.

(*b*) *Resection of nephric rudiment in the region cranial to the caudal end*

In the preceding experiments it was shown that, when the caudal end of the nephric rudiment was extirpated and the caudal growth prevented, there was no formation of the pronephric duct caudal to the region of operation. For control purposes a segment of nephric rudiment some distance from the caudal end was resected. If the development of the pronephric duct is due to the caudal growth of the end of the rudiment, a duct should be formed caudal to the region of operation. Seventeen such

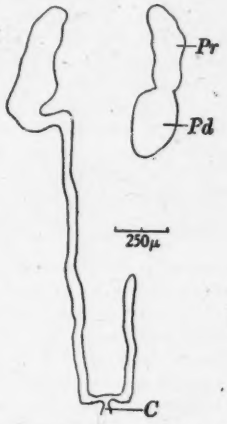

Fig. 3. Nephric system of *Bufo*, showing the normal development of the caudal segment of pronephric duct after resection of a segment of nephric rudiment in the region cranial to the caudal end of the rudiment. Lettering as in Fig. 1.

operations have been carried out, all of which showed a normal formation of the caudal portion of the pronephric duct. In fourteen cases the duct was absent in the operated region. Fig. 3 represents a diagram of such a case. The anterior portion of the duct became dilated while the posterior portion underwent a normal development. In the other three cases a single duct was formed. The portion in the region of operation was, however, much smaller than the cephalic and caudal parts. It is undoubtedly formed by regeneration as has been recorded in *Amblystoma* by Howland (1926).

(c) *Rotation of the caudal end of the nephric rudiment*

The above experiments furnish evidence that the nephric rudiment extends caudally by its own independent growth. Now the question arises, why in the normal development the extension of the rudiment is always caudal and not in other directions. Is there any relationship between the direction of growth and the antero-posterior axis of the embryo, or any influence exerted by the tissue at the ventro-lateral border of somites on the extension of the rudiment? In order to settle this question two series of experiments were devised. In one series the operations consisted of a rotation of the caudal end of the nephric rudiment. For the convenience of description the experiments may be divided into two groups according to the degree of rotation.

Rotation of 180°. At the ventral border of the somites a rectangular piece of tissue two or three segments in length was extirpated and grafted back with a rotation of 180°. The caudal end was included in the anterior segment so that after rotation it was carried one or two segments away from the cut end of the intact portion of the rudiment. It was found that in all thirty-three cases after excision of its caudal end the remaining portion of the rudiment was still capable of caudal growth. In spite of the reversal of the antero-posterior axis of the rotated tissue it extended into the tissue without any sign of disturbance though in some cases a reduction of size of the duct was observed.

As to the rotated portion of the rudiment, the original caudal limit extended cranially and in most cases fused end by end with the backward growing end of the cranial segment of the rudiment. Thus a single duct was formed. Sometimes the fusion of the two rudiments took place at a point other than their ends; in these cases two ducts were formed in the rotated region. This gives further evidence for the formation of the pronephric duct by an independent growth of the nephric rudiment, since if the duct is formed *in situ* by a proliferation of the somatopleure, only one duct would be formed in the rotated region. The cut end of the rotated rudiment was likewise capable of growth. It grew caudally and communicated with the cloaca or in a few cases with the gut.

To illustrate this type of experiment we have chosen three typical cases.

Case PD 93. The tissue ventral to somites 6 and 7 on the left side was rotated. It included the caudal end of the nephric rudiment at the level of the 6th somite. In sections a single duct was found on the operated side. The interest of this case lies in the fact that the duct in the rotated region changed its level to a position more ventral to the normal path. It can be seen clearly in Fig. 4, though the dorsal and ventral direction is not illustrated in the diagram. The same phenomenon was observed in the other series of experiments and will be fully discussed later.

Case PD 95. The operation was similar to the preceding case, the only difference being the origin of the rotated tissue which was taken from the ventral border of somites 6, 7 and 8 instead of 6 and 7 only, so that after operation the caudal end of the nephric rudiment was displaced in the region of the 8th somite. In sections it showed that the cranial portion of the rudiment grew backwards and fused with the rotated portion at the level of the 8th somite. The original caudal limit of the

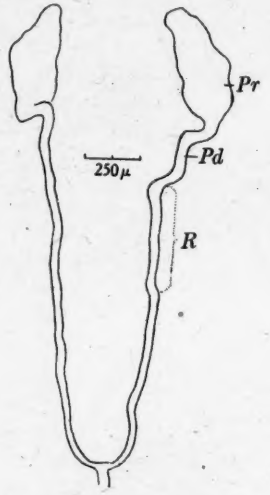

Fig. 4. Nephric system of *Bufo*, showing the formation of a single duct on the operated side after 180° of rotation of the caudal end of the nephric rudiment. Note the change of the level of the duct in the rotated region (*R*).

rotated rudiment grew cranially and ceased at the level of 7th somite, while the cut end extended caudally and united with the cloaca. Fig. 5 represents a diagram of the case. The two sections through the levels *a* and *b* are shown in Fig. 6 *a* and *b* respectively. The large medial duct on the right side of Fig. 6*a* is the pronephric duct derived from the rotated rudiment while the small lateral one comes from the cranial portion of the rudiment. Fig. 6*b* shows the fusion of the two ducts.

Case PD 113. The operation was similar to case PD 93. From Fig. 7 it is clear that the long pronephric duct on the operated side was formed by the caudal growth of the cranial portion of the nephric rudiment. In passing through the rotated tissue it became slightly dilated and then gained its normal size in the posterior segment. The

rotated rudiment had undergone no marked growth. It formed a short duct lying on the dorso-medial side of the long duct and united with the latter at the level of the 6th somite. In this case, therefore, both the excision of the caudal end of the rudiment and the reversal of the antero-posterior axis of the tissue in its path have no effect on the normal caudal growth of the nephric rudiment.

Rotation of 90°. A square piece of tissue about 2 somites in width, including the caudal end of the nephric rudiment was cut out, rotated through 90° and replanted in such a way that the original anterior edge of the piece was placed in the position of the original dorsal edge, so that the caudal limit of the rudiment was in the direction toward the

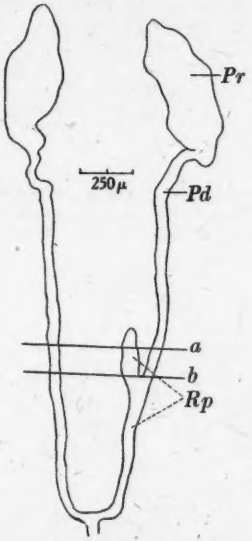

Fig. 5. Nephric system of *Bufo* in which the caudal end of the nephric rudiment was rotated through 180°. *a*, level of Fig. 6*a*; *b*, level of Fig. 6*b*; *Pr*, pronephros; *Pd*, pronephric duct; *Rp*, pronephric duct derived from rotated caudal end of the rudiment.

ventral side of the embryo. As the piece of tissue included a part of the somite material and lateral mesoderm in its dorsal and ventral edges respectively, the original path along which the rudiment normally grows was interrupted after rotation. In embryos so operated upon, the anterior portion of the pronephric duct underwent a considerable hypertrophy and ended blindly immediately cranial to the rotated tissue. The cut end of the rotated rudiment showed no sign of growth, while the original caudal limit grew ventrally and ceased at the cut edge. The growth of the rudiment was not really obstructed by tissues, since in most cases the duct lay immediately beneath the ectoderm where

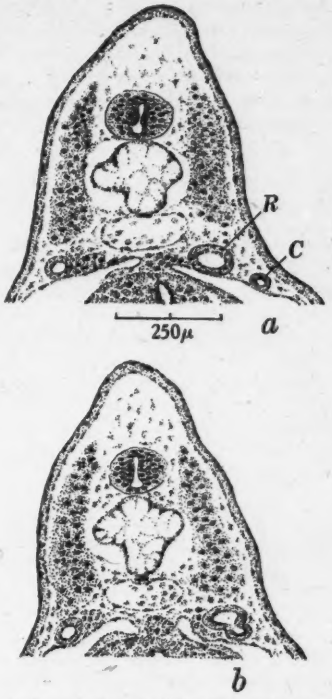

Fig. 6. *a. Bufo*, experiment of Fig. 5, showing two ducts in the rotated region. *R*, duct derived from rotated rudiment; *C*, duct derived from cranial portion of the rudiment. *b. Bufo*, experiment of Fig. 5, showing the fusion of the two ducts.

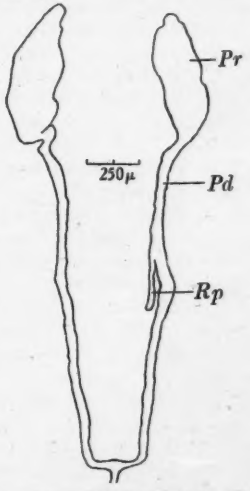

Fig. 7. Nephric system of *Bufo* in which the caudal end of the rudiment was rotated through 180°. *Rp*, duct derived from rotated rudiment.

no other tissues were found other than mesenchymal cells. It appears evident that the nephric rudiment cannot extend into or pass through the tissues other than the ventro-lateral border of the somites, which seem to be the predetermined path along which the nephric rudiment can grow.

(*d*) *Rotation of the tissue caudal to the caudal end of the nephric rudiment*

The above experiments of rotating the caudal end of the nephric rudiment furnish evidence that the caudal growth of the rudiment is controlled by a predetermined path without any relation to the antero-posterior axis of the organism. This evidence is supplemented by the present series of experiments where a piece of tissue caudal to the caudal limit of the rudiment was rotated. Experiments were performed on embryos at the stage where the nephric rudiment was confined to the anterior segments. As in the previous experiments, the rotated tissue was taken from the ventro-lateral border of the somites, including therefore, the predetermined path of the rudiment.

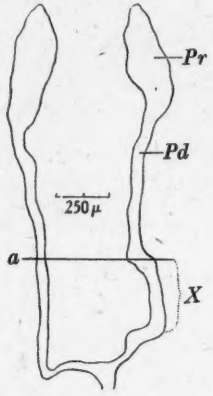

Fig. 8. Nephric system of *Bufo* in which a piece of tissue caudal to the caudal limit of the nephric rudiment was rotated through 180°. Note the change of level of the pronephric duct in the rotated tissue (*X*). *a*, level of Fig. 9.

In experiments where the tissue was rotated through 180°, the direction of the caudal growth of the rudiment varied in accordance with the mode of operation. If after rotation the path remained in its original level, the rudiment grew back along its normal level to the cloaca. Thus a straight duct resulted. On the other hand if the level of the path was shifted to a more ventral position, the growth of the nephric rudiment also changed its direction to the same level of the rotated path as it came into the rotated tissue. It grew upward again and returned to its normal level when it left the latter. This is best seen in Fig. 8. Actually that portion of the duct that lies in the rotated tissue and is designated in the diagram by a cross was situated in a more ventro-lateral portion. Fig. 9 is a section showing the downward movement of the duct. It appears, therefore, that the caudal extension of the rudiment must follow a certain definite path along the ventro-lateral border of the somites. In these cases and probably in those described in the preceding series of experiments, the level of the original path in the rotated tissue was changed after operation. This will not allow the rudiment to grow back in a straight line without changing its level.

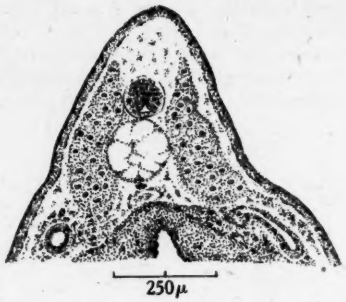

Fig. 9. *Bufo*, experiment of Fig. 8, showing the downward movement of the duct from the normal level to the rotated level of the path.

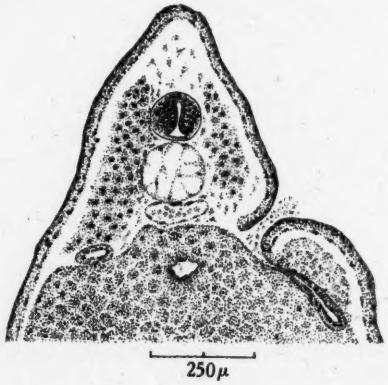

Fig. 10. *Bufo*, rotation of 90° of the tissue caudal to the caudal limit of the nephric rudiment. Section through the rotated tissue showing the downward growth of the pronephric duct.

The results of experiments of 90° rotation are of two kinds. In one group where the ventral cut edge of the rotated tissue was placed at the original

anterior cut edge, the original path in the rotated tissue being separated from that of the anterior segment by the lateral mesoderm, the rudiment ceased its caudal growth immediately cranial to the operated region. In the second group the dorsal cut by which the rotated piece was separated from the somites passed through the level just above the path. The tissue was then replanted in such a way as to place the original posterior cut edge in the original position of the dorsal edge, so that the rotated path was perpendicular to the path anterior to the operated region and connected with the latter to form a right angle. In embryos operated upon in this manner the nephric rudiment grew back normally until it reached the rotated tissue where it bent downward and continued growing ventrally along the rotated path (Fig. 10). These results again lead to the suggestion that the growth of the nephric rudiment is governed by a predetermined path regardless of the polarity of the latter.

SUMMARY AND DISCUSSION

We have demonstrated in the present experiments that in the embryos of *Bufo bufo gargarizans* and *Rana nigromaculata* when the caudal end of the nephric rudiment was removed and the caudal growth prevented by a piece of agar, there was no formation of the pronephric duct caudal to the operated region. If, on the other hand, the caudal end of the rudiment was rotated through 180°, two ducts were sometimes formed in the rotated tissues. It is apparent that the pronephric duct in anuran embryos, like that in urodeles, is formed by an independent caudal growth of the first formed nephric rudiment and not by a contribution from each individual segment. Whether the first rudiment is formed in a segmental manner as shown in *Amblystoma tigrinum* and *Triton taeniatus* by O'Connor is not demonstrated in our experiments.

The growth capacity of the rudiment is not limited to the caudal end, because after the removal of the latter the cut end of the remaining rudiment continues to grow. Howland (1926) reported that in *Amblystoma punctatum* at the tail-bud stage removal of two or five segments of the pronephric duct is usually followed by complete regeneration within a period of a few days. It is clear that the original growth capacity still persists in the late stage and is manifested in the process of regeneration. In normal development, however, the portion of the nephric rudiment cranial to the caudal end seemingly takes no part in the backward extension of the rudiment. This is proved by the resection experiment where removal of that portion of the rudiment does not exhibit any effect on the development of the caudal portion of the pronephric duct. The vital staining experiment of O'Connor pointed also to the same conclusion.

Attention must now be turned to the factors governing the caudal growth of the rudiment. Evidence derived from rotation experiments shows that the caudal extension of the rudiment is not controlled in relation to the axis of the body, because when the tissue caudal to the caudal end of the rudiment is rotated through 180°, the rudiment grows caudally without any sign of disturbance.

In normal development the rudiment extends backwards maintaining its position throughout at the ventro-lateral border of the somites. The results of our experiments show that this is the only path along which the rudiment can grow. If the path is obstructed the rudiment ceases to grow. On the other hand, if the level of the path is shifted, the growth of the rudiment also changes accordingly. The ventro-lateral border of the somites may be considered, therefore, as the predetermined path for the growth of the rudiment. It is not unreasonable to suggest that the surrounding cells of the path in some manner exert a stimulating influence on the growth of the rudiment, leading to an extension along a definite path so as to unite with the cloaca.

The final conclusion is that the pronephric duct of anuran embryos is formed by the caudal growth of the first-formed nephric rudiment with a close functional and topographical relationship with a predetermined path at the latero-ventral border of the somites. The development of the nephric system is, therefore, a very complex phenomenon, as Dalcq (1941) in his analysis has pointed out.

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IN MEMORIAM

EDWARD PHILIP STIBBE, F.R.C.S., *Professor of Anatomy, University of London*

1884–1943

PROF. E. P. STIBBE died at his home in Gerrards Cross on 24 July after a protracted illness. His passing has taken from the science of human anatomy a gifted exponent, and has left his many friends deeply conscious of their loss.

Born in 1884, he was educated at Allan Glen's School, Glasgow, and at Wyggeston School, Leicester, and received his medical training at Charing Cross Hospital. He qualified in 1908. For the next six years he worked abroad, first as Government Medical Officer at Fiji, then as district Medical Officer at Vosburg, South Africa. During the war years 1915–18 he was Medical Officer at the 1st Northern General Hospital. In 1919 he was elected Professor of Anatomy at the University of South Africa.

Returning to this country, he took his F.R.C.S. in 1925, and was demonstrator in anatomy successively at Durham, Liverpool, and University College, London. He was then appointed Senior Assistant to Prof. Wright at the London Hospital, where he spent five years. In 1935 he became University Reader in Anatomy and Sub-Dean and Medical Tutor at King's College. Three years later he was elected Professor.

Stibbe's earliest scientific work was a study of the internal mammary lymph glands with special reference to cancer of the breast. Thereafter he became interested in physical anthropology, and published observations on the heights and weights of certain South African tribes, with reference to disease-incidence. Then followed two interesting papers on the nictitating membrane, including a comparative study of the membrane in birds and mammals. His interests later passed to neurology and resulted in papers on the afferent components of the nerves of the eye-muscles, the anatomy of the intercostal nerves (with F. Davies and R. J. Gladstone), and two studies on the sensory root of the fifth nerve, in which he described a segregation of the fibres into spinal and principal nucleus fibres, and demonstrated the presence of scattered groups of nerve cells often quite close to the pons—an observation of considerable practical interest to neurological surgeons. The general conclusions from these last-named researches were summarized in a Hunterian lecture in the Royal College of Surgeons.

To the teaching of anatomy he also made considerable contributions. Among these may be specially mentioned his publication of *An Introduction to Physical Anthropology* (1930). He was also general editor of *Practical Anatomy by Six Teachers* (1932), and *Anatomy for Dental Students by Six Teachers* (1934). His wide and varied experience was reflected in the character of his lectures which were always interesting and wherever possible illustrated the application of his subject to clinical work. It is not surprising, therefore, that he won a considerable reputation as a teacher of undergraduate students.

Stibbe's affection for his pupils was a very real thing. The interest he took in their affairs, the sympathy with which he willingly helped to solve their individual problems, his lovable personality, and his kindliness and generosity must have put many everlastingly in his debt.

During the past two years his health gradually failed, but still further did he win the admiration of his friends for his patience and uncomplaining courage as he fought the battle with the disease which claimed his body but never could subdue his spirit. To his wife, who through all these months has by her own cheerfulness helped to strengthen and comfort him, we extend our sincerest sympathy.

T. N.